Wirksamkeit von Strategien zur Bekämpfung aeroterrestrischer Algenbiofilme

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1 Einleitung

Verbesserte Luftqualität, Klimaerwärmung, reduzierte Bauschadstoffe und effektive Wärmedämmung führten in den letzten Jahren zu einem erhöhten Auftreten von Mikroalgenbewuchs an Gebäuden. In einigen Stadtgebieten Norddeutschlands waren drei von vier Gebäuden mit den grünen und roten Biofilmen bewachsen (Venzmer & Messal 2003, Abb. 1.1). Besonders an nachträglich wärmegedämmten Gebäuden bildet sich infolge von Taupunktunterschreitung auf den in der Nacht auskühlenden Oberflächen Kondenswasser. Die entstehende Feuchtigkeit ist eine wesentliche Bedingung für das Wachstum von Mikroalgen.

Abb. 1.1: Fassade eines nachträglich wärmegedämmten Gebäudes mit deutlichem Algenbewuchs in der Rostocker Südstadt. Die Befestigungsdübel stellen Wärmebrücken dar, auf denen die Wasserverfügbarkeit gering ist. Sie bleiben daher algenfrei und sind als helle Punkte erkennbar.

Jedoch ist Algenbewuchs auf Oberflächen nicht erst eine Erscheinung der letzten Jahre. Aeroterrestrische Algen besiedeln schon seit vielen Jahrtausenden die Grenzfläche von Atmosphäre und Lithosphäre, etwa auf Steinen und Baumrinden (Gorbushina 2007). Hier entwickelten die Organismen vielfältige Anpassungen an ihren extremen Lebensraum. Dicke Zellwände, die Bildung von Osmolyten und mycosporinähnlichen Aminosäuren (MAAs) sowie eine Schicht aus extrapolymeren Substanzen (EPS) schützen die Zellen vor Austrocknung und extremer Strahlung (Karsten et al. 2007a, Karsten et al. 2007b, Gustavs et al. 2010). Ihre Anpassungen ermöglichen den Algen das Überdauern von ungünstigen Bedingungen, z.B. frostreichen Wintern oder trockenen Sommern. Aeroterrestrische Algen sind ubiquitär verbreitet und gelangen durch Luft-, Staub- und Regen-Immisionen auf die Fassaden

(Eckhardt 1994). So wurden beispielsweise im Rostocker Regenwasser bis zu 1000 Algenzellen je Milliliter gefunden (Schumann et al. 2004).

Auf Baumaterialien beeinträchtigen die Algenbiofilme durch ihre Verfärbungen nicht nur das Aussehen der Oberflächen, sondern können auch zu Schädigungen der Bausubstanz führen. Dabei sind eine Vielzahl von Schäden auch auf die mit den Algen assoziierten Bakterien und Pilzen zurückzuführen. Da Algen jedoch die Grundlage für weiteren Bewuchs bilden können, sind deren Einflüsse nicht von denen der Bakterien und Pilze zu trennen (Welton et al. 2003). Die Verfärbungen der Oberflächen durch Pigmente der Algen und Pilze werden durch die EPS, welche Staub- und Schmutzpartikel der Luft binden, verstärkt und verändern die Wärmeaufnahmekapazität des Materials (Warscheid & Krumbein 1994). Daneben können die Polymere der EPS strukturbildende Substanzen, wie z.B. Calcium, lösen (Scheerer et al. 2009). Durch wiederholte Austrocknung sowie Frost-Tau-Wechsel unterliegen die EPS einem ständigen Wechsel von Expansion und Kontraktion, welcher die Kristallstruktur der Baustoffe schwächt (Warscheid 1996). Aus dem Stoffwechsel der Organismen freiwerdende organische und anorganische Säuren reagieren direkt mit Bestandteilen des Baumaterials oder schädigen es über Salzkristall- oder Komplexbildung (Sand 1994). Hyphen und Filamente der Mikroorganismen dringen in Spalten und Poren des Baumaterials ein und können es bei Wasseraufnahme und Biomasseausdehnung aufsprengen (Crispim & Gaylarde 2005). Der durch Biofilme an Gebäuden entstehende wirtschaftliche Schaden ist beträchtlich. Für Deutschland beträgt er nach Schätzungen 2 bis 4 Milliarden Euro jährlich (Brill 1995).

Die Maßnahmen zur Bekämpfung von Algenwachstum an Gebäuden werden in konstruktive, physikalische und chemische Antialgenstrategien unterteilt. Konstruktive Maßnahmen richten sich gegen die Hauptursache des Bewuchses, eine hohe Wasserverfügbarkeit, und umfassen Mittel wie Dachüberstände, Wasserableitungen und passende Wärmedämmung (Brill 1995). Physikalische Maßnahmen verringern ebenfalls vor allem die Wasserverfügbarkeit an der Oberfläche sowohl durch hydrophobe - wasserabweisende - wie auch durch hydrophile - wasseranziehende - Beschichtungen (Cai et al. 2006, Solga et al. 2007). Dagegen beinhalten chemische Maßnahmen die Abtötung der besiedelnden Organismen. In der Regel werden biozide Wirkstoffe in die Materialien eingebracht, um die Vermehrung der Mikroorganismen an der Oberfläche zu verhindern. Der Einsatz von Bioziden ist aufgrund seiner möglichen Umweltgefährdung umstritten. So

werden Biozide aus dem Material ausgewaschen und gelangen in die Umwelt (Burckhardt et al. 2007). Chemische Wirkstoffe werden bei jeder Reaktion verbraucht und unter Umständen durch Sonnenstrahlung und Wärme abgebaut. Um eine lang anhaltende Wirkung zu gewährleisten, werden daher sehr hohe Biozidkonzentrationen eingesetzt. Dadurch erhöhen sich die möglicherweise in die Umwelt freigesetzten Wirkstoffmengen und die Gefahr der Schädigung von Non-Target-Organismen. Strengere rechtliche Regelungen wie die EU-Biozid-Richtlinie führten in den letzten Jahren dazu, dass zahlreiche Wirkstoffe als umweltgefährdend eingestuft und das Spektrum der verwendbaren Biozide deutlich eingeschränkt wurden.

Da konstruktive und physikalische Antialgenstrategien oft nur begrenzt wirksam sind und chemische Maßnahmen als potentiell umweltschädlich gelten, erhöhte sich der Druck auf Industrie und Forschung, neue Systeme mit verbesserten Eigenschaften zu entwickeln. Eine Innovation in der Bewuchsbekämpfung stellt die Photokatalyse dar, die sowohl chemisch als auch physikalisch wirkt und gleichzeitig verspricht, umweltfreundlich zu sein. Das Prinzip der Photokatalyse ist die durch Photonen, in der Regel ultravioletter (UV-) Strahlung, aktivierte Bildung von reaktiven Sauerstoffspezies (*Reactive Oxygen Species*, ROS) an Halbleitermetallen, wie z.B. Titandioxid (Thiruvenkatachari et al. 2008). Durch die Absorption von Photonen, deren Energie größer oder gleich der Energielücke zwischen Valenzband (vb) und Leitungsband (cb) des Halbleiters ist, werden Elektronen vom Valenzband ins Leitungsband angehoben (Mills & Le Hunte 1997, Abb. 1.2). Es bilden sich ein Elektronenüberschuss im Leitungsband sowie Elektronenlöcher im Valenzband. Diese Elektronen-Loch-Paare können mit Elektronendonoren und -akzeptoren (z.B. Wasser und Sauerstoff) reagieren. Die dabei gebildeten Radikale, in der Regel Hydroxylradikale, sind hochreaktiv und sollen Mikroorganismen oxidativ schädigen. Daneben bewirken photokatalytisch aktivierte Oberflächen eine starke Hydrophilisierung der Oberfläche, d.h. Wassertropfen breiten sich zu einem dünnen Film aus, der leicht ablaufen, abtrocknen oder aufgesaugt werden kann (Lackhoff 2002). Ein besonderer Umweltaspekt ist die katalytische Charakteristik, da der Halbleiter selbst sich nicht verbraucht und so dauerhaft wirkt. Photokatalytisch aktive Materialien werden als selbstreinigende Oberflächen bezeichnet, da keine sich verbrauchenden Wirkstoffe, sondern lediglich Sonnenstrahlung nötig ist, um ihre Aktivität aufrechtzuerhalten. Der Photokatalysator ist nicht wasserlöslich und die Gefahr der Auswaschung ist somit reduziert. Ihre Eigenschaften machen die Photokatalyse zu einer vielversprechenden Entwicklung

für den Einsatz als Antialgenstrategie an Baumaterialien. Seit einigen Jahren werden photokatalytisch aktive Materialien zu diesem Zweck bereits in Farben, Putzen, Dachziegeln, Fliesen und Gläsern verwendet und kommerziell vertrieben (Hashimoto et al. 2005, Fujishima et al. 2007). Jedoch ist eine Algenbewuchs verhindernde Wirkung dieser funktionellen Oberflächen noch immer nicht wissenschaftlich belegt.

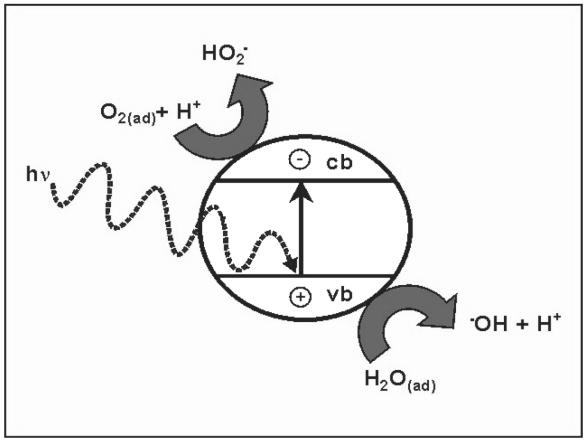

Abb. 1.2: Modell der Photokatalyse (Quelle: Lackhoff 2002).

Um die Wirkung und damit das Praxispotential von Antialgenstrategien zu bewerten, können zwei verschiedene Untersuchungsansätze unterschieden werden. Zum einen werden Untersuchungen durchgeführt, die das Ziel haben, eine mögliche Gefährdung für die Umwelt und ihre Organismen zu bestimmen. Zum anderen wird die Wirkung der Antialgenstrategie auf die Zielorganismen nachgewiesen und damit auch die Eignung, Biofilmbildung zu unterdrücken.

Erstgenannte ökotoxikologische Untersuchungen werden im Rahmen von gesetzlich vorgeschriebenen Prüfverfahren für chemische Bekämpfungsmaßnahmen durchgeführt. Dabei werden die Wirkungen auf das Wachstum und die Reproduzierbarkeit von Fischen (DIN EN ISO 7346), Daphnien (DIN 38412-11), aber auch Mikroalgen (DIN EN 28692, OECD 201) untersucht. Bei den verwendeten Algen handelt es sich um gut untersuchte aquatische Vertreter. Die Ergebnisse bestimmen das Gefährdungspotential eines Stoffes und entscheiden über seinen Einsatz als Wirkstoff. Dennoch sind aus diesen Untersuchungen kaum Rückschlüsse auf die Wirkung der Antialgenstrategien auf die Zielorganismen, aeroterrestrische Algen, und damit auf den Nutzen in der Praxis möglich. Die Tests der Zulassungsvorschriften können Wirksamkeitsnachweise nicht ersetzen. Zudem gibt es für physikalische Bekämpfungsmaßnahmen nach dem heutigen Stand überhaupt keine Zulassungsvorschriften.

Daher sind angepasste Verfahren für die Untersuchung der Wirksamkeit von chemischen aber auch physikalischen Antialgenstrategien notwendig. Die Anforderungen an diese Verfahren umfassen (1) die Arbeit mit Zielorganismen, um mögliche Schutzfunktionen zu berücksichtigen, (2) Inkubationsbedingungen, die sich an denen in der Praxis orientieren, und (3) die Beachtung der Eigenschaften der Antialgenstrategien selbst. Derzeit gibt es keine standardisierten Tests, um die Wirksamkeit von Antialgenstrategien an Baumaterialien zu untersuchen und jeder Hersteller von Farben, Putzen und Lacken erarbeitet sich eigene Testkriterien (Brill 1995). Dies führt dazu, dass Ergebnisse widersprüchlich und wenig vergleichbar ausfallen können. Um die Produkte jedoch zu bewerten und eine Schutzwirkung zu garantieren, müssen verschiedenen Studien zwingend standardisiert durchgeführt werden. International anerkannte Standards sollten im eigenen Interesse auch ein Ziel der Industrie sein.

Vielfach werden Antialgenstrategien in der Freibewitterung von Materialproben überprüft. Die realistischen Bedingungen erlauben eine gute Übertragung der Ergebnisse in die Praxis, jedoch sind oft erst nach Jahren Bewuchsvorhersagen möglich. Außerdem ist die Freibewitterung an nur einem Standort selten auf andere Gebiete übertragbar, da klimatische Faktoren das Algenwachstum hemmen und zu falsch-positiven Ergebnissen führen können.

In Laborversuchen werden Ergebnisse deutlich schneller erzielt. Die von der Industrie hier verwendeten Verfahren unterliegen jedoch einer Vielzahl von möglichen Fehlerquellen. Oft werden halbquantitative Methoden, wie z.B. der Agar-diffusionstest, angewendet, in denen die subjektiv bewertete Intensität von sichtbarem Bewuchs als Testparameter dient. Ein sichtbarer Bewuchs wird jedoch oft erst nach mehrwöchiger Inkubation erreicht. Durch Erreichen der stationären Wachstumsphase oder ungünstige Bedingungen, wie Selbstbeschattung, können Wachstumshemmungen dabei jedoch als biozide Wirkung fehlgedeutet werden.

Alternativ werden Methoden aus den oben bereits erwähnten Zulassungsverfahren für chemische Wirkstoffe angewendet und die Wirkung der bioziden Substanzen auf Algenwachstum in Suspension überprüft. Aus diesen Ergebnissen werden dann Algenbewuchs verhindernde Eigenschaften extrapoliert. Dabei wird jedoch die schützende Wirkung des Wachstums in Biofilmen nicht berücksichtigt. So fungieren die EPS des Biofilms vor allem als Diffusionsbarriere und trennen die zu schützende Oberflächen und die Zellen voneinander ab (Hall-Stoodley et al. 2004). Nicht zuletzt

werden aquatische Mikroalgen als Testorganismen verwendet. Mögliche Schutzfunktionen von aeroterrestrischen Mikroalgen, den eigentlichen Zielorganismen, bleiben unbeachtet. Aeoterrestrische Algen verfügen über strukturelle und funktionelle Anpassungen wie z.B. dicke Zellwände und Wachstum in Aggregaten (Karsten et al. 2007a, Gustavs 2010). Diese Eigenschaften schützen die Organismen nicht nur vor den extremen Lebensbedingungen ihres Habitats, sondern möglicherweise auch vor der Wirkung von Bekämpfungsmaßnahmen.

Das Beispiel der Photokatalyse macht die Notwendigkeit angepasster Verfahren für Wirksamkeitsnachweise besonders deutlich. Die photokatalytische Aktivität ist abhängig von der Intensität der UV-Strahlung (Benabbou et al. 2007). Dies erfordert zum einen die Anpassung der Strahlung an realistische Bedingungen im Habitat als auch die Kontrolle möglicher UV-Effekte auf die Algen. Zudem können Oberflächeneffekte, wie die der Photokatalyse, nicht in Suspension geprüft werden, da die aktiven Oberflächen mit den Zellen in Kontakt stehen müssen. Die Biomasse der Algen darf die durch Strahlung aktivierte Oberfläche nicht beschatten und sollte daher sehr gering sein. Dies hat zur Folge, dass die dünnen Algenbiofilme mit herkömmlichen Methoden nicht zu quantifizieren sind. Um die Wirksamkeit photokatalytischer Materialien zu bewerten, müssen neue Verfahren entwickelt werden, die diese besonderen Anforderungen erfüllen. Mit diesen Verfahren können dann wiederum auch andere physikalische und chemische Antialgenstrategien untersucht, bewertet und miteinander verglichen werden.

2 Ziele der Arbeit

In dieser Arbeit sollte zuerst die Frage beantwortet werden, wie die Wirksamkeit von Antialgenstrategien an Baumaterialien nachgewiesen werden kann. Dafür wurden die strukturellen und funktionellen Anpassungen aeroterrestrischer Algen an die extremen Bedingungen ihres Habitats und die Merkmale ihrer Lebensweise im Biofilm charakterisiert. Die Auswirkungen dieser Eigenschaften auf die Effektivität von Bekämpfungsmaßnahmen wurden analysiert und diskutiert. Daraus konnten Schlussfolgerungen für die Durchführung von Wirksamkeitsnachweisen abgeleitet werden. Der Fokus lag dabei auf der Photokatalyse als vielversprechender Strategie für algenfreie Oberflächen. Es wurden verschiedene Wirksamkeitsnachweise für Antialgenstrategien verglichen und ein Laborverfahren entwickelt, das die Eigenschaften der Zielorganismen, ihrem Habitat sowie auch der funktionellen Oberflächen berücksichtigt.

Zweites Ziel dieser Arbeit war die Untersuchung der Wirksamkeit verschiedener Antialgenstrategien, insbesondere der Photokatalyse. Ihre Wirkung auf die Vitalität aeroterrestrischer Algen in Labor und Freiland wurde analysiert, um die Wirksamkeit dieser Strategien zur Bekämpfung aeroterrestrischer Algenbiofilme an Baumaterialien zu bewerten.

3 Ergebnisse

Die Ergebnisse werden anhand von drei englischsprachigen Originalpublikationen präsentiert, die bereits veröffentlicht wurden.

Gladis F, Eggert A, Karsten U, Schumann R. 2010. Prevention of biofilm growth on man-made surfaces: evaluation of antialgal activity of two biocides and photocatalytic nanoparticles. Biofouling

Gladis F, Schumann R. 2011. A suggested standardised method for testing photocatalytic inactivation of aeroterrestrial algal growth on TiO_2-coated glass. International Biodeterioration & Biodegradation

Gladis F, Schumann R. 2011. Influence of material properties and photocatalysis on phototrophic growth in multi-year weathering. International Biodeterioration & Biodegradation

Den folgenden Artikeln sind kurze Zusammenfassungen in deutscher Sprache vorangestellt.

3.1 Prevention of biofilm growth on man-made surfaces: evaluation of antialgal activity of two biocides and photocatalytic nanoparticles

Um die Bildung von Algenbiofilmen an Baumaterialien zu bekämpfen, sollen potentiell umweltschädliche Biozide durch neue Antialgenstrategien, wie Photokatalyse, ersetzt werden. In dieser Arbeit wurde die Wirkung von zwei herkömmlichen Bioziden (Triazin und Isothiazolin) und photokatalytisch aktiven Nanopartikeln aus Zinkoxid auf die Vitalität aeroterrestrischer Mikroalgen verglichen. Als Modellorganismus diente eine aeroterrestrische Grünalge der Gattung *Stichococcus*. Aus dem Vergleich einer Auswahl von verschiedenen Vitalitätsparametern konnten letale und subletale Effekte der Antialgenstrategien bestimmt werden. Strukturelle Zellfunktionen (Membranintegrität, Chlorophyll *a*- [Chl *a*-] Gehalt, Autofluoreszenz des Chlorophylls) sind physiologischen Parametern (Wachstum, maximale Quantenausbeute, Primärproduktion) gegenübergestellt worden. Anschließend wurde die Eignung dieser Parameter in Wirksamkeitsnachweisen diskutiert.

Aus der Wirkung auf die verschiedenen Zellfunktionen konnte auf den Wirkmechanismus der Antialgenstrategien und ihr Potential für den Einsatz in der Praxis geschlossen werden. Obwohl das Herbizid Triazin effektiv Wachstum und photosynthetische Leistungsfähigkeit der Mikroalgen hemmte, wurden strukturelle Funktionen (z.B. Membranintegrität) von der EC 100, der geringsten Konzentration, die das Wachstum vollständig hemmte, nicht beeinträchtigt. Dieses Herbizid tötete die Algenzellen also nicht ab, sondern inaktivierte sie wahrscheinlich nur vorübergehend. Dies hat zur Folge, dass die Algen möglicherweise Resistenzen bilden und das Biozid unwirksam wird. Im Gegensatz dazu hatten Isothiazolin und photokatalytische Nanopartikel, welche durch geringe UV-Strahlung aktiviert wurden, gravierende Auswirkungen auf alle Leistungs- und Strukturparameter. Die breite Wirksamkeit von Isothiazolin und Zinkoxid gefährdet Non-Target-Organismen stärker. Besonders das wasserlösliche Isothiazolin kann leicht aus den Baumaterialien ausgewaschen werden. Die Eigenschaften der photokatalytischen Nanopartikel als sich nicht verbrauchende und damit nachhaltige Antialgenstrategie mit einer breiten Wirksamkeit nur unter UV-Strahlung versprechen ein hohes Potential für den Einsatz in Baumaterialien. Für eine Bewertung muss jedoch die Wirkung der Photokatalyse auf den Algenbiofilm untersucht werden.

Prevention of biofilm growth on man-made surfaces: evaluation of antialgal activity of two biocides and photocatalytic nanoparticles

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As algal growth on man-made surfaces impacts their appearance, biocides and surfaces with self-cleaning properties are widely used in the building and paint industries. The objective of this study was to evaluate the antialgal activity of two biocides (triazine and isothiazoline) and photocatalytic nanoparticles of zinc oxide (20–60 nm). An aeroterrestrial green, microalgal strain of the genus *Stichococcus* was chosen as the test organism. By comparing a set of different structural and physiological performance parameters, lethal and also sublethal (chronic) effects were determined. Even though the herbicide triazine effectively inhibited growth ($EC50 = 1.6\ \mu mol\ l^{-1}$) and photosynthetic performance, structural properties (eg membrane integrity) were unaffected at the EC100 ($250\ \mu mol\ l^{-1}$), hence this herbicide did not kill the algal cells. In contrast, and due to their multiple modes of action, isothiazoline and the photocatalytic nanoparticles (the latter activated with low UV radiation) severely impacted all performance and structural parameters.

Keywords: aeroterrestrial algae; growth prevention; effectivity tests; biocides; photocatalysis

Introduction

Growth of aeroterrestrial green algae on man-made surfaces such as roof tiles, building facades and concrete pathways is regarded as a biofouling problem (Gaylarde et al. 2003; Eggert et al. 2006; Karsten et al. 2007a). Whether these phototrophic organisms actively corrode a surface material, eg by the excretion of organic and inorganic acids, remains the subject of debate (Ortega-Calvo et al. 1995), but their discolouring effects together with deterioration and accelerated weathering are well known (Ortega-Calvo et al. 1995; Gaylarde and Morton 1999).

Aeroterrestrial green algae are exposed to extreme environmental conditions on man-made surfaces, and these organisms show several morphological and physiological adaptations to their habitat. A low surface to volume ratio, thick cell walls and formation of cell aggregates as observed for *Apatococcus* spp. and *Coccomyxa* spp. protect the cells against osmotic and desiccation stress, high temperatures and UV radiation (Karsten et al. 2007a; Rindi 2007). Additionally, these algae typically form assemblages (biofilms) which are embedded in a matrix of extracellular polymeric substances (EPS) secreted by the organisms (Barberousse et al. 2006). This biofilm matrix provides a protective microenvironment in the immediate vicinity of the algal cells (Ophir and Gutnick 1994). Thus, aeroterrestrial microalgae are typically well protected against environmental stress including chemical impacts of antialgal agents.

Prevention of algal growth on man-made surfaces is of particular economic importance and hence a number of different biocides are widely used. Triazines and isothiazolines are common classes of biocides, which are added to paints and plasters (Brill 1995). While triazines target very specifically the D1 key protein in the reaction centre of photosystem II (PSII), thereby blocking photosynthetic electron transport (Oettmeier 1992), isothiazolines interact with thiol-groups of amino acids, thus degrading various proteins with multiple consequences for cell metabolism (Collier et al. 1990). While the impact of triazine has been examined in many phototrophic organisms (eg Devilla et al. 2005), investigations on isothiazolines are mainly limited to human cells and tissues (eg Frosali et al. 2009) and so far, only a few phototrophs have been studied (Larsen et al. 2003).

A new trend in the building and paint industry is the application of photocatalytic surface coatings using their self-cleaning properties, primarily to remove unwanted organic pollutants (Hashimoto et al. 2005). Photocatalysis is defined as the acceleration of a photoreaction by the presence of a catalyst. The latter participates in and accelerates the chemical

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transformation of a substrate, itself remaining unaltered at the end of each catalytic cycle. During photocatalysis, free radicals, primarily hydroxyl radicals, are formed on the surface of semiconductors such as zinc oxide (ZnO), titanium dioxide and silicon dioxide by absorbed radiation energy, mostly UVA radiation (315–400 nm, Mills and Le Hunte 1997). Other reactive oxygen species (ROS), like superoxide and hydrogen peroxide, can be formed in secondary reactions. ROS are highly reactive and, at high concentrations, are extremely harmful for all organisms as essential molecules can be easily oxidised (eg peroxidation of lipids in cell membranes [Kiwi and Nadtochenko 2004]; degradation of polysaccharides impacting cell walls and EPS [Linkous et al. 2000]; degradation of whole cells [Kühn et al. 2003]). The nanostructure of semiconductor particles enlarges the effective surface and accelerates the rate of reaction. A beneficial effect of such nanoparticles is their inexhaustible catalytic character, which should result in long-term activity. While photocatalysis with titanium dioxide nanoparticles has already been applied to roof tiles, paints, concrete and glass panels (Hashimoto et al. 2005; Fujishima et al. 2007) and their deactivating effects on bacterial endospores (Lee et al. 2005), bacterial activity (Maness et al. 1999) and bacterial and algal adhesion (Gopal et al. 2004) are documented, the application of ZnO is still limited to the degradation of organic molecules (eg Gondal and Sayeed 2008). Furthermore, the direct effect of any photocatalytic particles on aeroterrestrial algae has not been tested so far.

Active agents have to be effective over a long period of time (the implied warranty of prevention is typically 5 years) and they must also be environmentally and materially compatible, ie must have a low water solubility and a high resistance against photolysis (Siebert 1994; Gaylarde and Morton 1999). To ensure whether approved, as well as active agents under development, meet the numerous demands, ecotoxicological investigations have to be performed with standardised methods (DIN 28 692, 1993; OECD 201, 2006). In these studies, aquatic algae (eg *Scenedesmus*) are the test organisms, but not aeroterrestrial target organisms, which have the specific adaptations referred to above. Thus, the applied methods may not be appropriate to evaluate the efficacy of the active agents under terrestrial conditions. To validate the performance of active agents for building materials, the development of microbial biofilms is observed on or in the vicinity of coated surfaces. Often, test pieces are embedded in agar or weathered outdoors in long lasting experiments over several weeks or even months. This methodological approach has several disadvantages, for example, misinterpretations of reduced growth rate during stationary growth phase. Consequently, there is an urgent need to develop fast and reliable bioassays to test the efficacy of active agents used in the building industry to prevent biofilm growth on man-made surfaces.

Although growth, a common activity parameter in ecotoxicological studies, integrates numerous types of impacts and is regarded as a sensitive parameter, even its complete inhibition does not automatically imply cell death because recovery is possible. To discriminate between dead and viable cells, fluorescent dyes (eg SYTOX® Green) can be applied that penetrate only the permeable membranes of dead cells. Using this approach, the minimum concentration of an active agent for complete mortality can be deduced. Another approach to evaluate the physiology of phototrophic organisms includes an analysis of the degree of pigment degradation (Louda et al. 2002) as well as photosynthetic performance using chlorophyll *a* (chl *a*) fluorescence-based parameters. The maximum quantum yield of photosystem II (F_v/F_m) has been used in numerous studies to quantify the activity of stress agents (Schreiber et al. 2002), as well a quantification of primary production (Wasmund 1989; Lin et al. 2005), although the latter has not so far been used in bioassays.

The aim of the present study was to evaluate a set of parameters to test the antialgal activity of three active agents, two biocides (triazine and isothiazoline) and one type of nanoparticles (ZnO). By comparing the results, lethal and sublethal (chronic) effects could be determined. Methods for testing antialgal activities are discussed.

Methods

Active agents

Three substance classes of active agents already in use or intended for use in coatings to prevent biofilm growth on man-made surfaces were included in this study: two biocides and one type of photocatalytic nanoparticle. The class of triazines was represented by Irgarol® 1051 (2-(*tert*-butylamino)-4-(cyclopropylamino)-6-(methylthio)-s-triazine, Riedel-de-Haën, analytical standard) and is termed 'triazine' below. The compound termed 'isothiazoline'was ProClin™ 950 (2-methyl-4-isothiazoline-3-one, Supelco, 9.5% solution in water. To prepare stock solutions of 1 and 10 mmol l^{-1}, triazine was dissolved in 5% aqueous ethanol and isothiazoline in distilled water. Stock solutions were autoclaved at 121°C for 20 min. The ZnO nanoparticles were obtained directly from the manufacturer (BYK Chemie GmbH) and were suspended in distilled water with a particle content of 49.5% (w/v) and a particle size of 20–60 nm. The concentration of ZnO was calculated by drying a

defined volume of the suspension on GF-filters at 60°C for 24 h. To prepare test suspensions of different agent concentrations, the stock solutions and suspensions were diluted with sterile algal cultivation medium (see below). To test whether either the triazine or isothiazoline was degraded during incubation, their concentrations were determined by HPLC at the start and the end of each experiment to determine the EC50 and EC100 (see below). Samples were centrifuged and analysed with a RP-C18-column (KNAUER, Hypersil 120 ODS, 5 μm, 250 × 4 mm) at a flow rate of 0.8 ml min^{-1} using two solvents: (A) 9:1 (v/v) methanol:water and (B) water. The gradient program was as follows: held at 80% A for 12 min, linear to 100% A over 5 min, held for further 5 min and completed to 80% A within 5 min. The retention times of triazine and isothiazoline were 9.0 min and 2.8 min, respectively. The compounds were detected by a diode array detector (G1315A, Agilent, Germany) at 225 nm and 280 nm, respectively.

Photocatalytic effects of the nanoparticles were initiated by UV radiation (UVR) using Q-Panel lamps (UVA-340 Lamp Q-Panel Co, USA, 3 W UVA m^{-2}, 0.15 W UVB m^{-2}) corresponding to the radiation regime in the natural habitat of aeroterrestrial algae at a north (shaded) facade of a building in Germany.

Culture conditions and application of active agents

The green algal test organism was isolated from a building facade in Rostock in northeast Germany (ROS 55/3) and was deposited at the Culture Collection of Algae (SAG) at the University of Göttingen (Germany) as strain SAG 2060. Its 18S rDNA identified this isolate as a *Stichococcus* sp. (Trebouxiophyceae, Karsten et al. 2005). This strain is a common aeroterrestrial biofilm species that has been intensively characterised with respect to its ecophysiological performance (Schumann et al. 2005; Eggert et al. 2006; Häubner et al. 2006; Karsten et al. 2007b; Gustavs et al. 2009a). Its robustness and fast growth make it a suitable test organism.

All tests were performed with algal cells cultured in modified Bolds Basal medium (MBBM: Bold 1949, modified by addition of triple nitrate concentration and vitamine solution) under standard conditions as described by Eggert et al. (2006) and with cells in the logarithmic growth phase, which was reached after 3–5 days. For growth measurements, batch cultures were grown in 30 ml disposable Petri dishes with cover lids (Licefa GmbH & Co KG, Germany). Three replicates at 13 different concentrations between 0.001 and 500 μmol l^{-1} of triazine and isothiazoline were tested. To measure viability, batch cultures were grown in 100 ml Erlenmeyer flasks at two concentrations (EC50 and EC100, for details see below). ZnO was investigated at 12 different concentrations between 0.03 and 44 mg cm^{-2}; each concentration was replicated three times. As nanoparticle suspensions were opaque, all assays were conducted on glass microfibre filters (diameter = 25 mm, GF/F, Whatman GmbH, Germany). For this, 500 μl of nanoparticle suspensions at the respective test concentration were pipetted onto the filters and dried for at least 12 h in a desiccator. After sterilisation under UVC radiation for 20 min, 500 μl of algal culture were pipetted onto each filter, corresponding to $\sim 10^6$ cells cm^{-2} and an initial autofluorescence of about 0.2 V (for details see below). To avoid desiccation of cells during incubations, filters were moistened daily with sterile medium.

The impact of half (EC50) and maximal (EC100) effect concentrations of triazine and isothiazoline (see below) on different structural and performance parameters were determined. In each experiment, agent-free incubations served as controls. The effects of ZnO nanoparticles with photocatalytic activity were investigated under UVR. Several controls were used: (1) incubations without nanoparticles under UVR (UVR control), (2) with nanoparticles without UVR to check for direct Zn^{2+} effects and (3) without nanoparticles and without UVR to evaluate filter incubations, to test for desiccation stress. Three replicates were analysed for each experimental condition after incubation for 4 days. Short term effects were measured only as chl *a*-based fluorescence parameters during the first hour of incubation (for details see below).

Growth response to active agent treatment

The growth measurements for the triazine and isothiazoline treatments were performed using an *in vivo* growth fluorimeter (Hansatech MFMS, UK) according to the technique of Gustavs et al. (2009b), which monitors increases of *in vivo* chl *a* fluorescence F_t over time as an indicator of biomass accumulation (Karsten et al. 1996). As the growth rate of *Stichococcus* sp. was high, fluorescence measurements were performed every 24 h for only 5 days. As the detection set-up for the *in vivo* growth fluorimeter is designed to measure chl *a* fluorescence in transparent media, a PAM-2000 fluorometer (Heinz Walz GmbH, Germany) was used to determine growth rates of the algal cells exposed to nanoparticles on the non-transparent GF/F filters. In this case, the initial *in vivo* chl *a* fluorescence (F_0) over time served as an indicator of biomass accumulation and measurements were performed according to Eggert et al. (2006), every 24 h for 5 days. After dark adaptation for 5 min, a 0.6 s far-red pulse (735 nm, intensity setting 7, *ca* 180 μmol photons m^{-2} s^{-1}) was given to fully oxidise the electron transport chain. F_0

was recorded with 600 Hz pulsed red measuring light (650 nm). The light intensity of the instrument was 7 and the gain 6. The optical fibre was always placed at a right angle 7 mm above the sample. Five measuring points were chosen per filter to account for heterogeneity of the algal cells and the F_0 values of each filter were averaged. A linear relationship between biomass and F_0 has been shown for the same *Stichococcus* strain in the range 100–1000 mV (Eggert et al. 2006).

The growth rate (μ) was estimated by fitting an exponential growth model to the data points. Based on these rates, inhibition rates were calculated (Equation 1) and concentration–response curves were fitted with the Hill model (Equation 2).

$$I[\%] = \frac{(\mu_C - \mu_T)}{\mu_C} 100 \quad (1)$$

with I = inhibition expressed as % change to control, μ_C = mean growth rate of control and μ_T = growth rate of treatment with the active agent.

$$E_C[\%] = \left(\delta + \frac{(\alpha - \delta)c^H}{EC\,50^H + c^H}\right) 100 \quad (2)$$

with E_c = inhibition expressed as % to control values at the effect concentration c of the active agent, δ and α = minimum and maximum inhibition, H = Hill slope and EC50 = 50% effect concentration (turning point of the curve).

At least 12 concentrations of each substance between 0 and 100% inhibition were used to calculate concentration–response curves. Higher growth rates than control values were considered as 0% inhibition, decreasing fluorescence over time was interpreted as 100% inhibition of growth. EC50 was derived from the concentration–response relationships. EC100 was the lowest test concentration, which stopped growth of the algal cells completely in all three replicates. No observed effect concentration (NOEC) was the highest test concentration, which did not affect growth.

Structural parameters

To analyse 'membrane integrity', cells were concentrated by centrifugation (5 min, 1560 g). Filters were washed in medium to suspend the cells. Cells with permeable membranes were stained with the fluorescent stain SYTOX® Green (Molecular Probes Inc, USA), which penetrates only permeable membranes and is a signal for dead cells (Veldhuis et al. 2001). The final concentration of the dye was 0.5 μmol l^{-1} and algal cells were incubated for 5 min. Stained cells were applied to a cover slip and counted with an inverted microscope Olympus IX 70 (Objective UPlan Apo 60/1.20 W × 1.5, Japan). SYTOX® Green fluorescence was excited by a blue filter set (U-MWB2). At the same time, additional weak transmitted light visualised cells without chl *a* and dye fluorescence allowing a total cell count. Cells with intact chl autofluorescence were prepared under the same conditions and counted using the same method by epifluorescence microscopy as for membrane integrity, but without staining. A minimum of 400 cells was counted four times for each replicate.

Chlorophyll content was detected by HPLC in 15 ml of algal suspension filtered onto GF/F filters (triazine and isothiazoline) and algae incubated on filters (ZnO nanoparticles). Pigments were extracted after mechanical homogenisation of filters with glass micro-beads for 2 × 3 min by dimethyl formamide (DMF) as the extraction solvent (Schumann et al. 2005). The HPLC method (Dressler et al. 2007) was used to separate chlorophylls and their degradation products. Pigments were determined by a diode array detector (G1315A, Agilent, Germany) at 436 nm (reference 550 nm). Reference standards were prepared from pure chl *a* of *Anacystis nidulans* (Sigma-Aldrich), which was dissolved in 100% acetone. To prepare standards of phaeophytine, chl *a* was acidified with 1 mol l^{-1} HCl (Brotas and Plante-Cuny 1996). The absorption spectra of all standards were read in a UV-VIS-Recording-Spectrophotometer UV 2401 PC (Shimadzu, Japan). From these spectra, concentrations were calculated using Lambert–Beer's law with molar extinction coefficients from Jeffrey et al. (1997). Pigment concentrations of the samples were calculated via single point calibration of the reference standards.

To determine the cell-specific chlorophyll content, cell numbers of glutaraldehyde-fixed samples (final concentration 1.2%) were determined in a Bürker hematocytometer. A minimum of 400 cells were counted four times using an Olympus BH-2 (Objective SPlan 20 0.46, Japan) microscope. As the abundance of cells on filters (nanoparticle treatment) could not be determined, the chlorophyll content was normalised to algal dry mass measured after drying at 60°C for 24 h.

Performance parameters

The chl *a* fluorescence-based parameter 'F_v/F_m', the maximum quantum yield of photosystem II of "dark-adapted" samples, was measured using a PAM-2000 fluorometer (Heinz Walz GmbH, Germany) with the same experimental set-up as described above for the growth measurements. To investigate the effects of triazine and isothiazoline, algal cells were filtered onto GF/F filters, incubated in 6-well cell culture plates

covered with test solutions. The experimental set-up with ZnO nanoparticles was not altered. Algae were darkened for 5 min and F_0 was measured with red measuring light pulses adjusted to F_0 of ~0.3 V and F_m was determined with an 800 ms completely saturating white light pulse (intensity = 6, ~9200 μmol photons m^{-2} s^{-1}). Short term effects of the active agents were detected within the first 30 min in intervals of 3 min. To record the same algal cells during a 60 min run, neither the position of the filter nor of the fibre optic was altered. To test the effect of ZnO particles, algal cells were filtered onto filters and were measured immediately (no photocatalytic effect). Subsequently, filters were exposed to UV radiation for 15 min, dark adapted for 5 min and measured again (with photocatalytic effect). This was repeated three times. Five points on each filter were recorded and averaged because it was impossible to place the filters accurately on the same position after UV incubation.

Inhibition of 'primary production' was measured as oxygen evolution of the algal cells by a new sensitive optode setup (Warkentin et al. 2007). This method uses optodes in the form of sensor spots (PreSens, Germany), which were installed inside of air-tied Plexiglass chambers (diameter 5 cm, height 2 cm). These were placed into a temperature-controlled water bath at 20°C (DC10 and K10; Thermo Haake GmbH, Germany). The samples were constantly stirred by a magnetic stirrer. After measuring cellular respiration in the dark for 30 min, the chambers were exposed for 30 min to the photon fluence rate of the culture conditions (35 μmol photons m^{-2} s^{-1}, Osram Lumilux Deluxe Daylight L58W/950, Germany) and oxygen concentrations were detected every 5 s. Thereafter, 1 ml samples of algal suspension were fixed with glutaraldehyde for counting later. Changes in oxygen evolution caused by the ZnO treatment were normalised to algal dry mass as described for the pigment analysis. Cellular respiration in the dark and net primary production in the light were summed to give gross primary production.

Statistical analyses

Statistical analysis of the data was performed using SPSS software (version 15.0). Significant differences in the inhibition of viability were assessed by 1-way ANOVA and a Tukey *post hoc* test at $p = 0.05$. Concentration–response curves were fitted by using GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego, California, USA). The goodness of concentration–response fits was expressed as r^2 and the 95% confidence band of the curves was calculated.

Results

Verification of the experimental approach

After treatment of *Stichococcus* sp. with triazine and isothiazoline for 4 days, the concentrations equivalent to the EC50 and EC100 concentrations in the medium were unchanged although many bacteria were found in samples at the higher triazine concentrations. Biocide-free controls were less contaminated by bacteria (data not shown). Since triazine is selective for photosynthetic organisms, bacteria from non-axenic algal cultures were promoted in these treatments.

Even though *Stichococcus* sp. SAG 2060 is an aeroterrestrial algal strain, incubation on filters with nanoparticles of ZnO reduced growth rates by 80% to 0.25 day^{-1} in comparison with the liquid cultures (Figure 1). However, the other viability parameters (membrane integrity, autofluorescence, F_v/F_m) were only marginally influenced by the treatment type.

The photocatalytic effects of ZnO nanoparticles needed to be activated by UVR. *Stichococcus* sp. strain SAG 2060 is well adapted to UVR as the effects of UVR exposure only were low. Only F_v/F_m dropped slightly (by 15%; data not shown). For the other parameters; membrane integrity, chlorophyll content, autofluorescence, primary production and growth, inhibition by UVR was not significant (data not shown). Also, nanoparticle treatment without UVR exposure only slightly affected the algal cells. The parameter F_v/F_m was inhibited by about 8% at the EC100. Hence, the effects of treatment with ZnO nanoparticles presented below are caused by photocatalysis and not by the ZnO itself. The effects of photocatalytically active ZnO nanoparticles were related to the control exposed to UVR.

Concentration–response relationships

All the substances tested inhibited growth of *Stichococcus* sp. SAG 2060. Figure 1 shows the increases of *in vivo* chl *a* fluorescence F_t over time as an indicator of biomass accumulation of the control, a medium and a high test concentration. However, these concentrations do not represent the EC50 and EC100 as they were calculated later from the concentration–response curves. While the control grew in liquid medium with $\mu = 1.1$ day^{-1}, the rate declined by 55% to 0.5 day^{-1} after application of 2.5 μmol l^{-1} triazine and the same concentration of isothiazoline. For both biocides, the initial fluorescence signal also decreased over time at 250 μmol l^{-1}, ie growth inhibition was 100%. Photocatalytic ZnO nanoparticles reduced growth rate by 66% from $\mu = 0.25$ day^{-1} to $\mu = 0.11$ day^{-1} at 1.3 mg cm^{-2} and growth was completely inhibited at 1.9 mg cm^{-2}.

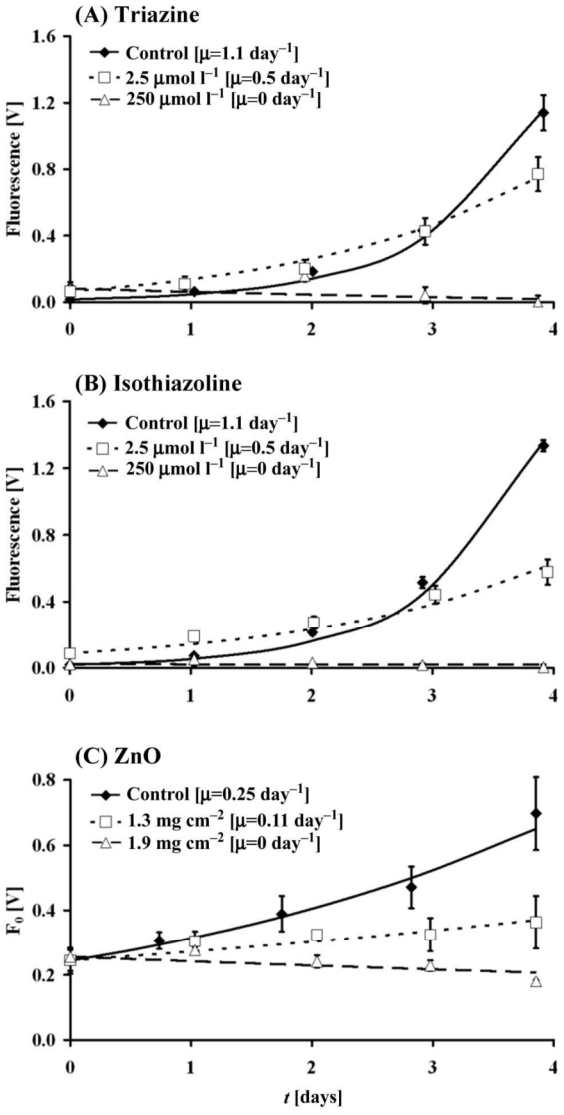

Figure 1. Growth of *Stichococcus* sp. with no active agent, a medium concentration and a high concentration. (A) Triazine. (B) Isothiazoline. (C) ZnO nanoparticles with UV induction. Measured values, fitted growth curves and calculated growth rates μ are shown. Means $\pm$ SD, $n = 3$.

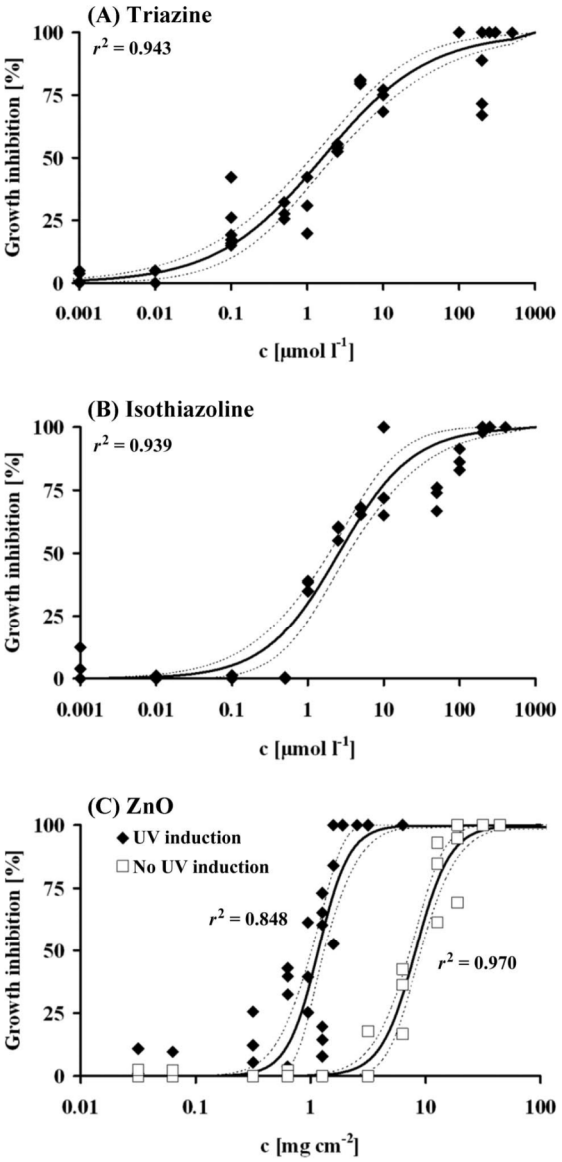

Figure 2. Concentration–response curves with measured (symbols) and fitted (solid line) growth inhibitions (%) of *Stichococcus* sp. after treatment with (A) triazine, (B) isothiazoline and (C) ZnO nanoparticles with UVR induction and without UVR induction. The 95% confidence intervals of the fitted curve (dotted lines) and coefficient of determination r^2 are shown. $n = 48$ for triazine, $n = 42$ for isothiazoline, $n = 48$ for ZnO nanoparticles with UVR induction, $n = 36$ ZnO nanoparticles without UVR induction.

Concentration–response curves of the three active agents were calculated from growth inhibition at all test concentrations (Figure 2). The slope of the concentration–response curve for photocatalytic ZnO nanoparticles was the highest at 3.2, while the curves of triazine and isothiazoline had a comparatively low slope with 0.6 and 0.9 (Table 1). The NOEC of triazine was 0.01 μmol l^{-1} while isothiazoline was less effective and induced first effects at concentrations >0.1 μmol l^{-1}. However, EC50 concentrations of the two biocides were similar (triazine: 1.6 μmol l^{-1}, isothiazoline: 2.6 μmol l^{-1}). For both, growth was completely inhibited at concentrations of 250 μmol l^{-1} (EC100). A photocatalytic effect was verified for the ZnO

Table 1. Characteristic effect concentrations (NOEC and EC100) and best-fit values (EC50 and Hill slope, mean ± SE) of concentration–response curves after the Hill model of *Stichococcus* sp. for triazine, isothiazoline and ZnO nanoparticles with and without UVR induction.

	Triazine (μmol l^{-1})		Isothiazoline (μmol l^{-1})		ZnO with UVR (mg cm^{-2})		ZnO without UVR (mg cm^{-2})	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
NOEC	0.01		0.10		0.06		1.30	
EC50	1.60	0.28	2.60	0.38	1.17	0.07	8.19	0.43
EC100	250		250		1.90		32.0	
Hill slope	0.62	0.06	0.89	0.11	3.22	0.60	2.88	0.34

nanoparticles as toxicity was significantly increased with UVR induction. The NOEC of the nanoparticles decreased from 1.3 mg cm^{-2} without UVR to 0.06 mg cm^{-2} with UVR induction. With UVR, EC50 of the nanoparticles was approximately eight times (8.2 *vs* 1.2 mg cm^{-2}) and EC100 was more than16 times higher than without UVR induction (32 *vs* 1.9 mg cm^{-2}).

Effects on structural parameters

All active agents at concentrations that lead to a 50% reduction in growth (EC50) did not or only slightly affected performance and structural parameters (data not shown). The few exceptions (membrane integrity of the nanoparticle treatment, F_v/F_m of the triazine-treatment) will be described separately. However, all agents in concentrations that inhibited growth completely (EC100) considerably affected the performance parameters (F_v/F_m, primary production), while the structure of the algal cells (membrane integrity, chlorophyll content, autofluorescence) was affected to different degrees by the agents.

Under control conditions, at least 99% of all cells had intact chloroplasts and membranes, because they emitted a red autofluorescence and only few cells were stained by the green fluorescent dye SYTOX® Green (Figure 3A). Even at EC100, triazine did not affect 'membrane integrity' (Figure 4A), but bacterial abundance increased as shown in Figure 3B. The ratio of dead cells increased to 38% at the EC100 of isothiazoline (Figures 3C and 4B). Photocatalysed ZnO nanoparticles at EC100 had intermediate effects with 16% dead cells (Figures 3D and 4C), but inhibition at EC50 was already comparatively high (25%, data not shown).

Pigment analysis by HPLC analysis separated chlorophylls from their main degradation products, ie phaeophytins, chlorophyllides and phaeophorbides. The chl *a* content ranged from 160 to 400 μmol per cell in log phase liquid cultures and from 2.3 to 5.9 μmol mg DW^{-1} on filters. Incubation with EC100 of triazine did not significantly alter the chlorophyll

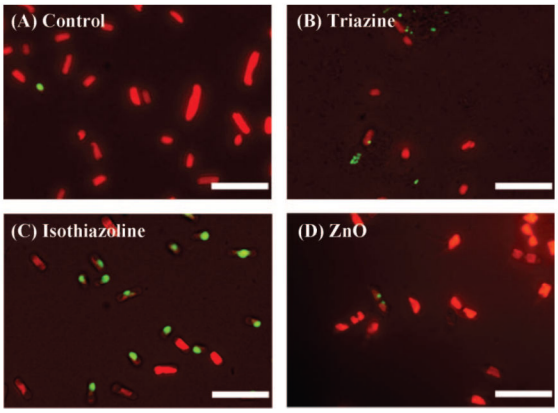

Figure 3. Viable (red autofluorescence of chlorophyll), chlorophyll degraded (pale) and permeable (green) cells of *Stichococcus* sp. stained with SYTOX® Green after incubation for 4 days in (A) control conditions and EC100 of (B) triazine (250 μmol l^{-1}), (C) isothiazoline (250 μmol l^{-1}) and (D) ZnO nanoparticles with UVR induction (1.9 mg cm^{-2}). White scale bars = 20 μm.

content (Figure 4A). Isothiazoline and nanoparticles at EC100 impacted cell-specific chlorophyll content to a similar degree by 80% and 67%, respectively (Figure 4B,C). The ratio of chlorophyll degradation products to chlorophylls did not change over time (data not shown). Cell-specific autofluorescence, as visualised by microscopy, confirmed the chlorophyll content. Cells with a bright chlorophyll autofluorescence amounted to >99% under control conditions (Figure 3A). After 4 days, triazine did not change the ratio of cells emitting a bright red autofluorescence even at EC100 (Figures 3B and 4A). In contrast, the EC100 of isothiazoline and nanoparticles resulted in a decrease of the ratio of cells emitting a bright red autofluorescence by 60% and 40%, respectively (Figure 4B,C). The affected cells appeared pale under blue excitation from scattered light (Figure 3C,D). The fluorescent dye SYTOX® Green was mainly detectable in such pale cells, which are regarded as damaged or even dead.

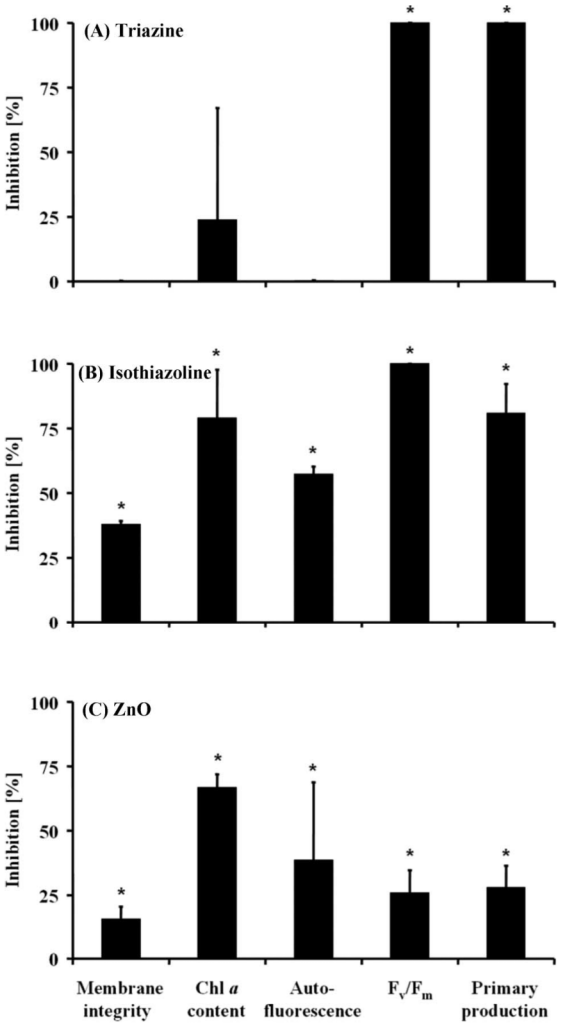

Figure 4. Inhibition of structural and performance parameters of *Stichococcus* sp. compared to control treatments detected as membrane integrity, chl *a* content, autofluorescence, F_v/F_m and primary production (gross production) after incubation for 4 days at EC100 of (A) triazine (250 μmol l^{-1}), (B) isothiazoline (250 μmol l^{-1}) and (C) ZnO nanoparticles with UVR induction (1.9 mg cm^{-2}). Means $\pm$ SD, $n = 3$. Asterisks represent significant differences from control ($p \leq 0.05$).

Effects on performance parameters

The maximum quantum yield F_v/F_m was 0.6–0.7 under control conditions (Figure 5). As triazine is known to impact photosynthetic electron transport very quickly, short term effects within the first 30 min were recorded in addition to long term effects over 4 days. F_v/F_m dropped rapidly in triazine-treated cells (A). Within 30 min, F_v/F_m decreased from 0.6 to 0.2 at EC100

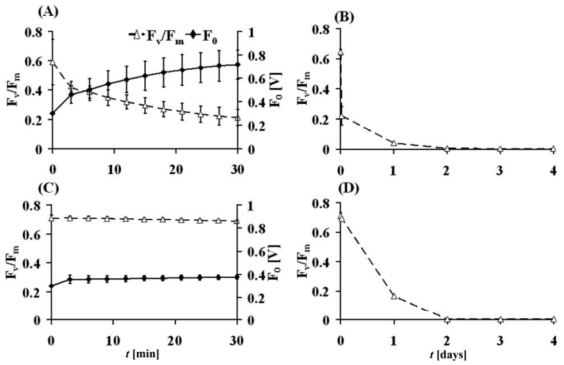

Figure 5. Short-term and long-term effects on the maximum quantum yield (F_v/F_m) (△) and the initial fluorescence F_0 (♦) of *Stichococcus* sp. during incubation for (A) 30 min and (B) 4 days with EC100 of triazine (250 μmol l^{-1}) and during incubation for (C) 30 min and (D) 4 days with EC100 of isothiazoline (250 μmol l^{-1}). Means $\pm$ SD, $n = 3$.

caused by rising F_0 from 0.2 to 0.7 mV at constant F_m values. After 1 day, F_v/F_m approached a minimum level of 0.04 (B) and the cells did not subsequently recover. However, at EC50, rapid effects were similar to EC100, but F_v/F_m recovered to 0.6 within 1 day (data not shown). Even though isothiazoline and ZnO nanoparticles at EC100 did not affect F_0 and F_v/F_m during the short-term, F_v/F_m decreased slowly during the first day (isothiazoline: Figure 5C,D; ZnO: data not shown). After 4 days, F_v/F_m was completely inhibited in the triazine- and the isothiazoline-treatments, while the nanoparticles only caused a small inhibition by 10% (Figure 4).

Cellular respiration in the dark and gross primary production of controls at 35 μmol m^{-2} s^{-1} was 1.3 and 4.3 pmol O_2 h^{-1} $cell^{-1}$, respectively, in suspensions and 170 and 300 μmol O_2 h^{-1} g^{-1} DW when measured on filters (Figure 6). All agents strongly inhibited photosynthetic oxygen evolution under irradiation, ie net primary production. Due to the large number of bacteria (see above) in the triazine-treatments, respiration in the dark increased considerably in all these samples (215 pmol O_2 h^{-1} algal $cell^{-1}$). As oxygen consumption remained high in the light (260 pmol O_2 h^{-1} algal $cell^{-1}$), gross primary production was always zero. Isothiazoline caused a small (30%) reduction in cellular respiration in the dark, but net primary production was inhibited almost completely (95%). Thus, gross primary production was inhibited by 80% to 0.8 pmol O_2 h^{-1} $cell^{-1}$. ZnO nanoparticles increased respiration moderately and reduced gross primary production by 28%. This means that net primary production was inhibited almost completely by 90%.

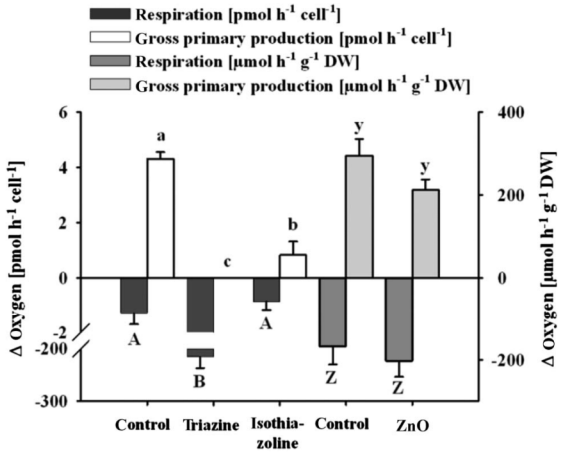

Figure 6. Respiration (black and dark grey, respectively) and gross primary production (white and light grey, respectively) (Δ pmol O_2 h^{-1} $cell^{-1}$, and Δ µmol O_2 h^{-1} g^{-1} DW, respectively) of *Stichococcus* sp. after incubation for 4 days under control conditions and EC100 of triazine (250 µmol l^{-1}), isothiazoline (250 mmol l^{-1}), control conditions under UVR and EC100 of ZnO nanoparticles (1.9 mg cm^{-2}). Means and SD of respiration and gross primary production are shown as the sum of respiration in the dark and net primary production under irradiation of the culture conditions (35 µmol photons m^{-2} s^{-1}) ($n = 3$). Letters represent significant differences between the treatments ($p \leq 0.05$).

Discussion

How to evaluate antialgal activity of active agents

This study applied a different methodological approach to test antialgal activity that counterbalanced some of the disadvantages of conventional tests for ecological risk assessment and verification of efficacy. The ecological risks of active agents are typically tested in aquatic organisms (eg fish, *Daphnia*, freshwater algae) according to the OECD test guidelines. However, aeroterrestrial algal strains are required to evaluate their efficacy in preventing biofilm growth on man-made surfaces, as these organisms exhibit specific adaptations to their harsh terrestrial environment that are not present in aquatic species (Karsten et al. 2007a). The strain of *Stichococcus* used in this study is a common biofilm species and its robust and fast growing attributes qualify it as a test organism (Schumann et al. 2005; Eggert et al. 2006; Häubner et al. 2006; Karsten et al. 2007b; Gustavs et al. 2009a). *Stichococcus* spp. are important members of aeroterrestrial habitats throughout the world (McKnight et al. 2000; Miller et al. 2008; Novis et al. 2008). Other important aeroterrestrial green algae, like *Apatococcus* spp., are difficult to isolate and grow too slowly and not sufficiently reliably for toxicity tests. In addition, they form aggregates, which cause a high heterogeneity between replicates from self-shading, the latter impacts all pigment- and photosynthesis-based parameters. Other important aeroterrestrial species of the *Chlorooidium* genus (Darienko et al. Forthcoming 2009) are also reported to be suitable test organisms. However, *Stichococcus* strains are considered to be the best choice in view of the published data on ecophysiology and stress tolerance.

So far, two approaches are used to screen the efficacy of antialgal agents: outdoor weathering with natural infection pressure or agar inhibition tests in the laboratory with aquatic species (see Briand 2009 for a review of test methods for aquatic organisms). Both tests require a visible biofilm that forms not earlier than after 1–2 weeks. All performance and structural parameters measured in this study can be obtained quickly (hours to 5 days). However, it is not only the economy of time that makes the alternative approaches more advantageous as long-term approaches have several disadvantages: (1) sublethal toxicity remains unrecognised, ie the degree of sublethal growth inhibition cannot be detected; (2) biocidal effects have to be separated from effects of solvents and other ingredients in the coating; (3) contamination with fungi and bacteria in non-sterile outdoor experiments can influence the results; (4) active agents may be degraded by photolysis or microorganisms in long-term experiments (Callow and Willingham 1996; Okamura et al. 1999); (5) algae may reach stationary growth phase and form dormant cells (eg spores or akinets) which can be misinterpreted as inhibition of growth. "Old" biofilms contain at least some, if not most of the cells in the stationary growth phase, which results in heterogeneous and unreliable stress responses (eg oxidative stress response [Sigaud-Kutner et al. 2002]); (6) the main problem of all tests requiring a visible phototrophic biofilm is the increasing self-shading with age (Liehr et al. 1990). Within a thick biofilm with a chl *a* content of 6 mmol cm^{-2}, intrinsic light decreases by 90% (Barranguet et al. 2004). Next to light, these biofilms have steep chemical gradients of oxygen, carbon dioxide and nutrients (Stewart and Franklin 2008). Since biocides are applied to surfaces, a vertical concentration gradient is formed, thus cell layers closest to the interface are most affected. These are the cells that receive the least light, carbon dioxide, and nutrients and are therefore least resistant to toxic agents. Limitation of light and physico-chemical gradients in the biofilm can be excluded in this study as incubation times were short and the chl *a* content amounted to a maximum of 11 µmol cm^{-2}. However, all biofilm responses to active agents are a mixture of multiple abiotic factors (and gradients) and the

physiological state of the organisms present. Therefore, assay results are always difficult to extrapolate to natural outdoor conditions on man-made surfaces.

The best measure of toxicity of antialgal agents

NOEC and EC50 are most commonly used in (eco)toxicity tests, because they describe those concentrations without or with low danger, respectively, for the test organisms. Based on the EC50 of the dose response curves in this study, triazine (EC50 = 1.6 μmol l^{-1}) and isothiazoline (EC50 = 2.6 μmol l^{-1}) are assessed as rather toxic for aeroterrestrial green algae. The NOEC for triazine was an order of magnitude lower than that of isothiazoline, most likely due to its specificity for photosynthesis. However, the Hill slopes of the dose response curves of both biocides were smaller than a Hill slope of 1.0, which is the slope of a standard curve for a one-site-binding agent (triazine: slope = 0.62; isothiazoline: slope = 0.89). Thus, *Stichococcus* cells must be able to compensate, to a certain degree, for the effects of the two biocides. In contrast, the Hill slope for the ZnO nanoparticles was very steep (slope = 3.22) for the aeroterrestrial *Stichococcus* strain and a number of other green algal isolates (data not shown), and hence the toxic effect of these photocatalytical nanoparticles must be regarded as very high.

However, inhibition values calculated from dose response curves are often criticised because they depend on the choice of test concentrations and the applied regression model (Isnard et al. 2001). Even though the EC50 is discussed as the most suitable measure for risk assessment (Isnard et al. 2001), the evaluation of active agents to prevent algal growth on man-made surfaces rather requires an EC100 effect concentration. EC100 ensures that all or at least enough cells are permanently inhibited (in growth). Unfortunately, the EC100 determined in this study (two biocides: 250 μmol l^{-1}; photocatalytical ZnO nanoparticles: 1.9 mg cm^{-2}) are difficult to compare with other active agents as this measure is only rarely reported in toxicity tests (Utgikar et al. 2001; Illmer and Mutschlechner 2004).

The choice of the most suitable functional or structural parameter

While the herbicide triazine has only one specific target site and only one mode of action, the two other agents affect cellular structures and/or functions in multiple ways. Triazine is believed to enter the cells very quickly by diffusion, and specifically blocks photosynthetic electron transport (Oettmeier 1992). As expected, triazine affected only the performance parameters (F_v/F_m, primary production), while the structural parameters were not impacted. As the rapid decrease of F_v/F_m of the *Stichococcus* strain was accompanied by an increase in F_0, triazine chronically damaged the photosynthetic apparatus similar to high light effects (Osmond et al. 1994). However, the fluorescence signal arises only from viable cells containing active chlorophyll, while dead cells with degraded chlorophyll reduce the absolute fluorescence intensity, but do not necessarily impact fluorescence ratios, as F_v/F_m. Chlorophyll degradation caused by secondary effects of triazine seems to be species specific. Similar to *Stichococcus*, at the EC100 no chlorophyll degradation was found when the cyanobacterium *Synechococcus* sp. was treated with triazine, while pigments were degraded in triazine-treated cells of the marine prymnesiophyte *Emiliana huxleyi* (Devilla et al. 2005).

Isothiazoline has multiple modes of action (eg Gorsuch et al. 2009) and thus affects both performance and structural parameters. The photocatalytic nanoparticles generate ROS (Gondal and Sayeed 2008), hence this active agent also has multiple target sites, but primarily damages cellular structures. Accordingly, isothiazoline and the photocatalytically active ZnO nanoparticles significantly inhibited all examined parameters in the *Stichococcus* strain. The effects on the performance parameters (F_v/F_m and primary production) were much stronger for isothiazoline than for the nanoparticles.

The photocatalytic activity of ZnO has been demonstrated in other studies (eg Gondal and Sayeed 2008) and was confirmed in this study. Even though zinc ions affect photosynthetic electron transport (Rashid et al. 1994) and inhibit enzymes associated with chlorophyll synthesis (Miao et al. 2005), the effect of the nanoparticles without UVR induction was only marginal. *Stichococcus* spp. have been reported to be exceptionally resistant to metals (Pawlik-Skowronska 2000). Although photocatalytically generated hydroxyl radicals are highly reactive (Hashimoto et al. 2005), algae have various strategies to cope with oxidative stress (Arora et al. 2002). It is also important to consider that ROS are normal by-products of a number of metabolic pathways and algae have the capacity to remove ROS by antioxidant compounds or a high activity of antioxidant enzymes (Arora et al. 2002). This may explain the relative insensitivity of the performance parameters (F_v/F_m and primary production) at effective concentrations of the ZnO nanoparticles that strongly inhibited growth.

It is important to emphasise that most parameters (growth, F_v/F_m, primary production, chlorophyll content) represent the average response of the population. Only membrane integrity and autofluorescence are cell-specific parameters giving the ratio of affected

and unaffected cells. Furthermore, even complete growth inhibition or the lack of performance (eg no primary production) is not equivalent to mortality (membrane integrity). This result was confirmed for triazine, ie all cells were potentially viable, but showed a complete inhibition of physiological performance. This differentiation is very important when aiming to prevent biofilm growth on man-made surfaces, especially over a long term. Algae can not only become dormant, resistant or repair damages, but can also use alternative substrate and energy sources. For instance, many algae can utilise external organic carbon sources and grow heterotrophically or mixotrophically when photosynthetic electron transport is specifically impacted by triazine (Lewitus and Kana 1995). Also *Stichococcus* sp. SAG 2060 showed increased cellular respiration in the dark after the addition of glucose (data not shown). Since cellulose is an important component of paint binders, the cellulolytic activity of biofilm algae themselves would be of interest. Even if they cannot use the polymers, there will be associated bacteria and fungi, of which at least some should be able to cleave the sugar monomers. Accordingly, when biocides and especially triazine are incorporated into paints and plasters, they may not always be very effective in controlling the survival of algal populations over a long period of time. Even though the impact of isothiazoline and the ZnO nanoparticles on membrane integrity and autofluorescence was much more severe, the active agents killed only 20–40% of the *Stichococcus* cells. Additionally, triazine, in common with almost all other biocides is known to leach out from paints and plasters in high rates (Heath et al. 1996) and is easily degraded by sunlight (Okamura et al. 1999). For building facades that are exposed to weathering and high solar radiation, this leads to a considerable loss of biocidal activity over time. If active agents are no longer present (by degradation or washing out) or cannot reach the target cells (covered by dirt or dead cells), there are most likely enough surviving cells to recolonise the surface (Solomon et al. 1996). These are the reasons why most active agents are applied at much higher concentration than EC50 or even EC100 on man-made surfaces.

Future perspectives

The potential impacts of any photocatalytic nanoparticles in the environment are still unknown and literature on this subject is still emerging (Handy et al. 2008). However, in contrast to water soluble triazines and isothiazolines, nanoparticles can be fixed more effectively in the coating matrix and the threat of leaching out into the surrounding environment is low, thereby resulting in lower ecological risks. However, a number of questions regarding their cellular effects remain unknown. For instance, do externally formed radicals penetrate into cells, for example, hydrogen peroxide is reported to be the only ROS that diffuses across membranes (Mallick and Mohn 2000). It is possible to quantify intracellular ROS by fluorescent dyes, however this method is criticised for its inaccuracy (Jakubowski and Bartosz 2000). Moreover, diffusion of radicals may be obstructed by mucoid substances secreted within a biofilm or be taken up by cells in the close vicinity of the photocatalytic surface, while more distant cells remain protected.

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3.2 A suggested standardised method for testing photocatalytic inactivation of aeroterrestrial algal growth on TiO_2-coated glass

Die Photokatalyse gilt als vielversprechende Strategie zur Bekämpfung von Algenbewuchs an Baumaterialien. Dennoch ist ihre Wirkung auf aeroterrestrische Algenbiofilme bisher nicht wissenschaftlich belegt. Daher wurde in dieser Studie ein Laborverfahren entwickelt, mit dem die Wirksamkeit von photokatalytischen Materialien auf Algenbiofilme nachgewiesen werden kann. Die Testmaterialien waren kommerziell erhältliche, photokatalytisch beschichtete Gläser und unbeschichtete Referenzgläser. Der Kompromiss aus naturnahen und das Wachstum fördernden Inkubationsbedingungen induzierte die Bildung von fest auf den Materialien haftenden Biofilmen. Durch die Kombination der Vitalitätsparameter Wachstum und Membranpermeabilität war eine mögliche Wirksamkeit der Antialgenstrategien sensitiv nachweisbar. Die drei Grünalgenisolate *„Chlorella" luteoviridis* (SAG 2196), *Coccomyxa* sp. (SAG 2040) und *Stichococcus* sp. (SAG 2060) waren geeignete Modellorganismen. Sie sind typische Vertreter der aeroterrestrischen Gemeinschaft und wuchsen bei hohen Luftfeuchtigkeiten und geringer UVA-Strahlung auf den Prüfkörpern. Dagegen war das Isolat *Apatococcus lobatus* (SAG 2096), welches zu den häufigsten gefundenen Algen an künstlichen Hartsubstraten zählt, nicht in der Lage, sich unter den hier verwendeten naturnahen Wachstumsbedingungen zu vermehren. Mit der vorgestellten Methode lassen sich auch andere Antialgenstrategien untersuchen. Durch die Standardisierung des Verfahrens wären die Ergebnisse zukünftiger Untersuchungen vergleich- und reproduzierbar.

In dieser Arbeit konnte jedoch keine Wirksamkeit der photokatalytisch beschichteten Materialien gegen aeroterrestrische Algenbiofilme nachwiesen werden. Obwohl die chemische Aktivität als Methylenblauabbau messbar war, wurde die Vitalität der Algen nicht inhibiert. Durch ihre Anpassungen an ungünstige Lebensbedingungen, wie z.B. die Bildung dicker Zellwände und EPS sowie das Wachstum in Zellaggregaten, scheinen die Zellen aeroterrestrischer Grünalgen auch vor den photokatalytisch gebildeten Hydroxylradikalen geschützt zu sein. Um die Diskrepanz der photokatalytischen Wirkung auf Algenzellen in Suspension und Biofilm zu klären, sollte die Wirksamkeit auf natürliche Algenbiofilme untersucht werden.

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A suggested standardised method for testing photocatalytic inactivation of aeroterrestrial algal growth on TiO_2-coated glass

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ABSTRACT

Aeroterrestrial green algae form conspicuous biofilms on man-made surfaces. The self-cleaning properties of photocatalytic coatings prevent their growth and can probably replace biocides. The aim of this study was to develop a laboratory method to investigate the efficiency of photocatalytic materials against algal growth. Two algal isolates (*"Chlorella" luteoviridis*, SAG 2196, and *Coccomyxa* sp., SAG 2040) functioned well as model organisms because they grew on almost all test specimens at 100% humidity and low UVA radiation. With these species, we examined algal growth prevention using photocatalytic glass. No effects on algal growth were detected, although the coated surfaces were photocatalytically active and degraded methylene blue. Because their cells are protected well against photocatalytically generated hydroxyl radicals, aeroterrestrial algae survive various stress factors. Nevertheless, the newly developed experimental design may be useful for assessing the biological function of other photocatalytic materials or stress factors.

1. Introduction

Aeroterrestrial green algae colonise man-made surfaces such as roof tiles, concrete and building façades, where they form conspicuous biofilms. Within extrapolymeric substances (EPS), algal cells are protected against physical stress (e.g., desiccation, changes in salinity, temperature and UV radiation) (Decho, 2000). Once established, biofilms become more diverse and form complex microbial biocoenoses that include cyanobacteria, heterotrophic bacteria and fungi (Gaylarde and Morton, 1999). Established biofilms are mechanically stable and can grow rapidly to a thickness of >100 μm. Biofilms damage substrates by chemical and physical alterations (Gorbushina, 2007), although the active functions of algae in these processes are still being discussed (Ortega-Calvo et al., 1995). However, bacteria and fungi excrete organic and inorganic acids that alter the chemical structure of materials (Gómez-Alarcón et al., 1994; Gaylarde et al., 2001). Furthermore, EPS can solubilise components of the building materials and damage the substrate by repeated wetting and drying cycles (Scheerer et al., 2009 and references therein). In addition, filamentous microorganisms may penetrate the material and detach particles (Ortega-Calvo et al., 1991). After all, algal biofilms affect surface appearance by discolouration and cause the adherence of dirt particles.

The prevention of algal biofilms on man-made surfaces is of particular economic interest. Biocides are widely used to cope with microbial growth. However, these chemicals may be washed out or can be photolytically and microbially degraded (Callow and Willingham, 1996; Burkhardt et al., 2007). Furthermore, biocides are harmful to the environment, as dissolved and dispersed fractions accumulate in soils and waters (Burkhardt et al., 2007). Structural measures (e.g., roof overhangs) may reduce water availability (which is the most-important factor for aeroterrestrial algal growth) at the façade surface but are not applicable or effective everywhere (Gladis and Schumann, 2011).

New functional (i.e., self-cleaning) surfaces are applied to building materials and protect surfaces from pollution. The commercially most-important development is photocatalysis. Photocatalysis is defined as the formation of positive holes in the valence band and an electron in the conduction band by photon absorption at a semiconductor catalyst surface (Thiruvenkatachari et al., 2008). The positive holes directly oxidise pollutants or water to produce free radicals, primarily highly reactive hydroxyl radicals. Furthermore, photocatalysis changes the water wettability of the surface as it becomes superhydrophilic (Hashimoto et al., 2005). Titanium dioxide (TiO_2), activated by UVA radiation (315–400 nm), is the most often applied photocatalyst (Mills and LeHunte, 1997). Photocatalysis is already applied to roof tiles, paints, concrete and glass panels (Hashimoto et al., 2005; Fujishima et al., 2007).

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The advantage of photocatalysis over biocides is its inexhaustible catalytic character, which results in long-term effectiveness. Photocatalytic surfaces are classified as environmentally friendly, as they operate using only sunlight and rainwater and without using any chemicals (Hashimoto et al., 2005).

The efficiency of photocatalytic degradation for oxidising (and degrading) various chemical compounds has been thoroughly investigated (Mills and LeHunte, 1997) and both simple and complex organic and inorganic molecules are mineralised with high activity. In addition to photocatalysis in suspensions, this oxidising process has been applied to degrade harmful molecules in the air such as NO_x (Cassar, 2004). Additionally, photocatalysis has been shown to be effective for inactivating organisms such as bacteria, viruses and yeasts (Mills and LeHunte, 1997 and references therein) and to have limited effects for some fungi, protozoa and algae (Matsunaga et al., 1985; Otaki et al., 2000; Sichel et al., 2007). On the other hand, photocatalysis has been suggested for wastewater treatment processes in which pollutants and organisms are mineralised or inactivated (Thiruvenkatachari et al., 2008; van Grieken et al., 2009). Applications, such as antimicrobial surfaces for hospitals, have also been evaluated and show promise (Page et al., 2009).

Self-cleaning surfaces for buildings accomplish both mineralisation of chemical compounds from the air and from dust and inactivation of biofilm-forming microorganisms. The hydrophilic surface properties cause rainwater to spread into a thin film, which supports debris wash-off by rainwater. Although such self-cleaning materials are already commercially available, their efficiency for inhibiting biofilm formation is still not verified. Photocatalytically coated roof tiles exposed to long-term weathering did not show any growth-preventing activity (Gladis and Schumann, 2011), although laboratory assessments of photocatalytic nanoparticles detected inhibition of algal growth by photocatalysis (Gladis et al., 2010). To explain such discrepancies, consistent and reproducible tests must be developed and must include sufficient positive (good growth without anti-algal effects) controls for UVA effects. Any microbicidal activity of photocatalysis against bacteria or fungi cannot be extrapolated to aeroterrestrial algal growth because the photosynthetic apparatus of many algae contains extraordinary defence mechanisms against radicals. Moreover, experimental conditions for phototrophic growth, such as lamp properties and media, are completely different from conditions for heterotrophic growth. Established toxicity tests for chemicals are also not applicable for photocatalytic surfaces, as such tests are adapted to test suspensions and not to test biofilms.

The aim of this study was to develop a test system for photocatalytic efficiency that could show results within a few weeks. The resulting method should be transferable to other materials and is suggested as a standard method. The exact choice of growth-promoting near-natural conditions allows for conclusions regarding the anti-algal potential of photocatalytic surfaces. This study examined commercially available photocatalytic glasses and assessed their efficiency against algal growth.

2. Experimental

2.1. Materials

The photocatalytic materials were 4×4-cm shards of TiO_2-coated glass (Pilkington Activ™ self-cleaning glass, Pilkington Holding GmbH, Germany). The TiO_2 layer had a thickness of ca. 15 nm on 4-mm soda-lime silicate glass. Mills et al. (2003) described this material in detail and suggested it as a reference material for semiconductor film photocatalysis. Uncoated glass shards (soda-lime silicate glass, Pilkington Holding GmbH, Germany) served as controls.

2.2. Organisms and culture conditions

Four aeroterrestrial green algae isolated from roof tiles and building façades were investigated in this study (for details see Gustavs et al., 2010). The isolates *"Chlorella" luteoviridis* (SAG 2196) and *Coccomyxa* sp. (SAG 2040) were chosen because they grow rapidly under 100% air humidity (Gustavs et al., 2010). *Stichococcus* sp. (SAG 2060) has been intensively characterised with respect to its ecophysiological performance and its suitability as fast-growing test organism (e.g., Häubner et al., 2006; Gustavs et al., 2010; Gladis et al., 2010). *Apatococcus lobatus* (SAG 2096) is a common alga in aeroterrestrial biofilms and was isolated from a façade in Japan. Nevertheless, it grows slowly in suspension and 100% air humidity (Gustavs et al., 2010). For cultivation in suspensions, algae were grown in modified Bolds Basal Medium (Bold, 1949, modified by addition of triple nitrate concentration and vitamin solution) under standard conditions as described by Eggert et al. (2006). Conditions included a photon flux density (PFD) of 35–40 μmol photons m^{-2} s^{-1} (Osram L36W/12-950, Germany) of photosynthetic active radiation (PAR, 400–700 nm) and a Light/Dark-rhythm of 16/8 h at 21 +/− 1 °C. Cultures in 250 ml Erlenmeyer flasks were aerated with filtered air and reached log-phase within 5 d for *"Chlorella" luteoviridis* and *Stichococcus* sp. and 10 d for *Coccomyxa* sp. and *A. lobatus*.

For each experiment, six photocatalytically coated and six uncoated shards lying in 9 cm petri dishes were investigated (Fig. 1). A total of 12 shards were incubated under a combination of photosynthetic active radiation (PAR) and ultraviolet radiation (UVA), so that three replicates were analysed for each of four experimental treatments. The first treatment with TiO_2-coated glass under PAR and UVA was used to detect photocatalytic effects. Uncoated glass under the same radiation served as a negative control for any photocatalytic effects and a positive control for UVA. TiO_2-coated glass under PAR, but without UVA, was used to detect effects due to UVA, while uncoated glass under PAR reflected any coating and surface effects. Radiation scenarios were realised using a combination of daylight lamps (Osram L36W/12-950, Germany), Q-Panel UVA-340 fluorescent tubes (Q-Panel, Cleveland, OH, USA) and cut-off filters. Under the PAR treatment, petri dishes were covered with a 395-nm cut-off filter foil (Folex PR, Folex, Germany). For the elimination of UVB, a 320-nm cut-off foil (Ultraphan URUV, Digefra, Germany) was applied for the UVA treatment. Applied radiation intensity (Table 1) corresponded to natural conditions on shaded building sites in summer (North Germany). In all treatments, the light/dark-rhythm was 16/8 h at 21.5 +/− 1.5 °C. A wet sponge in each petri dish kept air humidity at 100%. All shards were activated or pre-incubated without algae under these conditions for 3 d.

All tests were performed with cells in the logarithmic growth phase, which were gently centrifuged (5 min, 1560 g) to increase inoculum biomass. One ml of algal culture with about 10^6 cells ml^{-1} was pipetted onto all glass shards and allowed to stand for one hour to promote adhesion of algae. Algal growth was supported by adding diluted medium (1:10) to prevent osmotic stress by evaporation. In

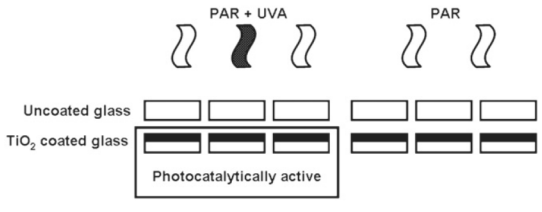

Fig 1. Schematic view of the experimental setup.

Table 1
Incubation designs with description of liquid supply, shard positioning and applied radiation intensity.

Incubation design	Liquid supply	Shard positioning	Radiation intensity
A	Sprinkled with diluted medium every 24 h	Plane	PAR: ~45 μmol photons $m^{-2} s^{-1}$ UVA: ~4 W m^{-2}
B	Sprinkled with diluted medium every 24 h	45°	PAR: ~45 μmol photons $m^{-2} s^{-1}$ UVA: ~4 W m^{-2}
C	Submerged into diluted medium for 30 min every 24 h	45°	PAR: ~45 μmol photons $m^{-2} s^{-1}$ UVA: ~4 W m^{-2}
D	Sprinkled with diluted medium every 24 h	45°	PAR: ~45 μmol photons $m^{-2} s^{-1}$ UVA: ~7 W m^{-2}

total, algae were incubated under four different conditions, each of which differed in liquid supply, positioning and radiation (Table 1). For the incubation design A, shards lay on a flat plane and were sprinkled with about two ml of sterile-filtered medium (1:10) every 24 h. To simulate more realistic conditions, shards were positioned at a 45° angle so that sprinkled medium (1:10) ran off their surfaces (design B). For design C, shards were completely submerged in medium (1:10) for 30 min per day to prolong water uptake. Afterwards, they were again positioned at 45°. Finally, incubation design D was similar to B but with increased UVA radiation to investigate possibly enhanced photocatalytic or UVA effects.

2.3. Biofilm quantification

After one, four, seven and ten days, the amount biomass on each shard was quantified with an inverse epifluorescence microscope (Olympus IX 70, Objective Uplan Apo 20x/0.70 × 1.5 extra magnification, Japan). Chlorophyll *a* autofluorescence was excited with green light (filter cube U-MWG2). Twenty to thirty microphotographs (260.8 × 208.6 μm^2) per sample, and evenly distributed over the shards, were taken with a camera (Olympus SIS Color View 12) and the software AnalySIS Pro 3.02 (Olympus Soft-Imaging GmbH, Germany). All camera settings (e.g., exposure time, RGB gain and intensity control) were kept constant within each experiment. One pixel had a resolution of about 0.2 × 0.2 μm. Shards were rinsed with medium (1:10) to remove free cells and the camera was focussed on the shard surface with a <1-μm depth of field so that only the adhered biofilm algae were included. The red fluorescing biofilm area was quantified from all micrographs with the software AnalySIS Pro 3.02. The threshold values for RGB channels were adjusted manually for each photograph to include all fluorescing pixels and to exclude the background. The extent of the biofilm-covered area was calculated in relation to the whole investigated area. Because growth on surfaces was slow, a linear growth model was used (Fig. 2).

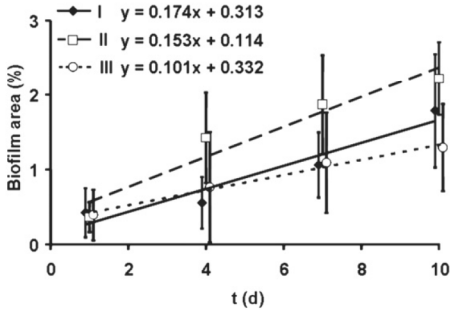

Fig. 2. Increase in biofilm area (%) of the three replicates of *Coccomyxa* sp. (SAG 2040) on uncoated shards under UVA in incubation design A. Means are shown +/− SD ($n = 20$). Lines and equations are a linear regression.

At the end of each experiment, the membrane integrity of the adhered algae was analysed. Shards, which were previously rinsed with medium (1:10), were submerged in the fluorescent-stain solution SYTOX® Green (Molecular Probes Inc, USA). This solution penetrates only permeable membranes and is a signal for dead cells (Veldhuis et al., 2001). The final concentration of the dye was 0.5 μmol l^{-1} and algal cells were incubated for 5 min. The proportion of stained algae was determined by counting a minimum of 400 cells four times for each replicate with an inverted microscope Olympus IX 70 (Objective Uplan Apo 60/1.20 W, Japan). SYTOX® Green fluorescence was excited by a blue filter set (U-MWB2).

2.4. Cell-specific viability

To more closely analyse algal viability, *Stichococcus* sp. was inoculated onto coated and reference shards with plastic frames glued onto their surfaces. This created a circular area of 9.6 cm^2 with a height of 1 mm. Three replicates of each shard type were pre-incubated for 24 h under a combination of 4 W m^{-2} UVA and 45 μmol $m^{-2} s^{-1}$ PAR (Light/Dark 24/0 h). Nine hundred and sixty μl of algal suspension from logarithmic cultures were pipetted into each well on the glass shard so that the frames were completely filled. The samples were then incubated for 48 h. Thereafter, algae were resuspended and subsamples were taken to analyse membrane integrity, oxidative stress and enzyme activity. For this process, cells were concentrated by centrifugation at 20 °C (5 min, 1560 g).

Membrane integrity was investigated according to biofilm analysis with the fluorescent-stain SYTOX® Green (final concentration 0.5 μmol l^{-1}). Oxidative stress was examined with the fluorescent-stain CM-H_2DCFDA (Molecular Probes Inc, USA), which labels cells with intracellular-reactive oxygen species and intact esterase activity. Cells were suspended in 20 μmol l^{-1} staining reagent (dissolved in DMSO) for 4 h at 40 °C. Algae were washed three times with medium and were counted as for membrane integrity under blue excitation. As a positive control for the staining procedure, algae were treated with 10 mmol l^{-1} H_2O_2 for 30 min. By this process, oxidative stress was detected in 95% of the algae. As a control for the permeation of the substrate and the intracellular esterase activity, the fluorescent-stain CMFDA (Molecular Probes Inc, USA) was used with a final concentration of 25 μmol l^{-1} to label algal cells with intact esterase activity.

2.5. Chemical activity

As it is a standard method to determine the activity of photocatalytic coatings (DIN 52980, 2008), the photocatalytic formation of hydroxyl radicals was measured as degradation of methylene blue. This method was developed after recommendations by Tschirch et al. (2008). Photocatalytically coated and uncoated materials were activated according to Fig. 1. Shards in petri dishes were covered with

deionised water and pre-incubated for 3 d with continuous agitation under 4 W m^{-2} UVA and 45 μmol m^{-2} s^{-1} PAR (light/dark 16/8 h). Thereafter, methylene blue was added at a final concentration of 10 μmol l^{-1}. The solution supernatant was <1 mm so that radiation attenuation by the methylene blue itself was minimised. Absorption was measured in a UV–VIS-Spectrophotometer UV 2401 PC (Shimadzu, Japan) at the absorption maximum of 660 nm for methylene blue and corrected for particle scattering at 750 nm. The first samples were taken after 1 h when degradation became linear. Three additional subsamples were measured every two hours. Methylene-blue concentrations were calibrated within a concentration range of 1–10 μmol l^{-1} at 6 points. Because degradation rates of uncoated shards under UVA were the highest of all of the controls (without photocatalytic activity), photocatalysis (chemical activity) was calculated by subtraction of UVA methylene-blue degradation.

Algal biofilms can minimise the amount of UVA intensity that reaches the photocatalytic coating as well as the transport of radicals to the supernatant. Therefore, the chemical activity of biofilm-covered shards was measured as described above but with six replicates. The shards were covered by *"Chlorella" luteoviridis* grown under design A for four weeks and were rinsed carefully to ensure that biofilm was adhered to the shard and to minimise the amount of free-floating algal cells in the methylene blue. Biomass on the shards was calculated via image analysis of the microphotographs after measuring the methylene-blue degradation.

2.6. Statistical analyses

Statistical analyses were performed using SPSS software (version 15.0). Fixed-factor effects of both the incubation design and the growth-rate treatment were analysed using two-way ANOVA. The significance of the main effects of the two factors (i.e., the effects of treatment averaged over the incubation design and *vice versa*) as well as the interaction effects of the incubation designs and treatments were calculated (level of significance: $p = 0.05$). Differences between treatments were analysed using simple main-effects analysis. Significant differences among treatments were further analysed by pairwise comparisons using Bonferroni adjustment of the type 1 error. The analysis of cell-specific viability and chemical activity was performed using one-way ANOVA and a Tukey *post hoc* test at $p = 0.05$.

3. Results

3.1. Choice of model organisms

To select suitable biofilm-forming model organisms for testing growth prevention on surfaces, the growth capacities of four aeroterrestrial algae on control shards were evaluated. Algal growth on control shards was considerably slower than growth in suspensions. The median for all incubation designs for *"Chlorella" luteoviridis* was only 0.01 d^{-1}, which corresponds to a doubling time of 69 d (Fig. 3). Nevertheless, the upper 75th percentile was 0.12 d^{-1} (doubling every 6 d). These higher growth rates were detected for growth under the incubation design A. Other incubation designs reduced algal growth. Positioning at 45° (incubation designs B and D) even suppressed growth, as more than half of the growth rates were negative.

Stichococcus sp. did not grow on most shards. Most (75%) of the growth rates were <0.05 d^{-1} (doubling every 14 d). On two-third of the shards, growth was suppressed to negative rates. Again, growth rates were highest (with a median of 0.13 d^{-1}) for incubation design A. On these shards, *Stichococcus* sp. established a biofilm whose area increased during incubation with a growth rate of at least 0.05 d^{-1} (doubling every 14 d). However, highly variable

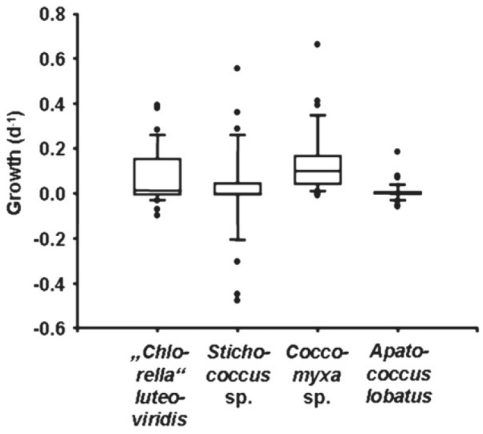

Fig. 3. Growth (d^{-1}) of controls of investigated algal isolates pooled from four incubation designs. Shown are the 25th and 75th percentiles as boxes with medians as lines, the 10th and 90th percentiles as error bars and dots as outliers ($n = 36$).

growth rates between replicates were conspicuous for *Stichococcus* sp. The maximum growth on shards was detected for the isolate *Coccomyxa* sp., with a median rate of 0.1 d^{-1}. Growth rates of this isolate were >0.06 d^{-1} even for the rather unfavourable exposure design B. Only incubation design D did not increase algal biomass (median 0.02 d^{-1}). Incubation design A promoted the most rapid and stable growth of *Coccomyxa* sp., as all control shards had growth rates between 0.10 and 0.22 d^{-1} (doubling time 3 d). In contrast, *A. lobatus* grew slowly or not at all on shards. The median for all incubation designs was only 0.0003 d^{-1}. Even for incubation design A, highest growth rates of *A. lobatus* were <0.01 d^{-1}.

After 10 days, biofilms of *Coccomyxa* sp. were largest (two-third of the shards grew by more than 1%) and thus were visible as a thin biofilm. The biofilm area of *Coccomyxa* sp. on control shards was 1.6% on average, while it was 1.2 and 1.0% for *"Chlorella" luteoviridis* and *Stichococcus* sp, respectively. In contrast, *A. lobatus* did not build visible biofilms (only 2 shards had biofilms of an area larger than 1%).

In conclusion, only *"Chlorella" luteoviridis* and *Coccomyxa* sp. grew stable on control shards without anti-algal surfaces. Thus, these two algal isolates are suitable to test the growth-preventing efficiency of photocatalytically coated surfaces. The following analyses were therefore conducted with these two algae.

3.2. Influence of incubation design

Water is the main growth-influencing factor for aeroterrestrial algal growth (Häubner et al., 2006; Gladis and Schumann, 2011). The four incubation designs differed in water availability. Shards under incubation design A maintained a thin liquid film throughout the incubation, while there was medium run-off from shards immediately after they were positioned at a 45° angle (incubation designs B, C and D). During the first hours shards were still wet, but after 24 h, surfaces were dry (except in the case of PAR-coated hydrophobic shards where drops remained).

Growth under the investigated incubation designs was significantly different for both types of algae ($p < 0.01$, Table 2). While growth for incubation design A was highest (and statistically significant) for *"Chlorella" luteoviridis*, *Coccomyxa* sp. grew fastest under design C. Nevertheless, this design was not suitable for growth tests on surfaces because the variation in growth rates was

Table 2
Parameters of the two-factorial ANOVAs for growth of *"Chlorella" luteoviridis* (SAG 2196) and *Coccomyxa* sp. (SAG 2040) on shards. Growth means were divided into subgroups via the Tukey *post hoc* test ($p \leq 0.05$).

Alga	*n*	Significance level	Incubation design		
			Subgroup 1	Subgroup 2	Subgroup 3
"Chlorella" luteoviridis	12	$p < 0.01$	B ($\mu = -0.01$ d^{-1}) C ($\mu = 0.00$ d^{-1}) D ($\mu = 0.08$ d^{-1})	A ($\mu = 0.16$ d^{-1})	
Coccomyxa sp.	12	$p < 0.01$	B ($\mu = 0.07$ d^{-1}) D ($\mu = 0.01$ d^{-1})	A ($\mu = 0.16$ d^{-1}) B ($\mu = 0.07$ d^{-1})	C ($\mu = 0.31$ d^{-1})

high. For instance, growth rates of the two test algae varied between negative and particularly high values for control shards (-0.1 to 0.66 d^{-1}) making the results difficult to assess. The lowest growth rates were seen for the designs B and D. In contrast, design A promoted the growth of both *"Chlorella" luteoviridis* and *Coccomyxa* sp. Hence, the most appropriate incubation design to examine photocatalysis on algae was incubation design A. Although it was excluded as suitable test organism, *Stichococcus* sp. also grew under this incubation design. Thus, the effects of photocatalysis will also be analysed for this isolate.

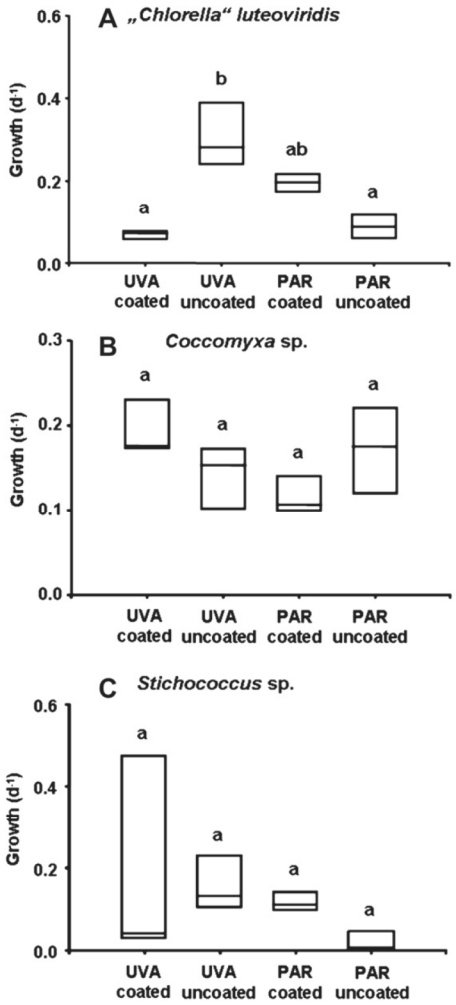

Fig. 4. Growth (d^{-1}) of A) *"Chlorella" luteoviridis* (SAG 2196), B) *Coccomyxa* sp. (SAG 2040) and C) *Stichococcus* sp. (SAG 2060) on photocatalytically coated shards under UVA and controls (Table 1) under incubation design A. Shown are minimums and maximums as boxes and medians as lines ($n = 3$ for each box). Letters represent significant differences between the treatments ($p \leq 0.05$).

3.3. Effects of photocatalysis

Growth inhibition was attributed to photocatalysis if it was significantly different from growth inhibition on other controls. Growth of *"Chlorella" luteoviridis* was highest on uncoated shards under UVA with an average of 0.30 d^{-1}. This isolate grew slowly (with a growth rate of 0.07 d^{-1}) on photocatalytically coated shards under UVA; however, it was not significantly different from the controls under PAR (Fig. 4A). Growth-inhibiting effects of photocatalysis on this alga were not observed. Supporting this result, the growth of *Coccomyxa* sp. was similar on all investigated treatments (mean 0.16 d^{-1}, Fig. 4B). *Stichococcus* sp. grew with rates between 0.48 and 0.001 d^{-1} (Fig. 4C). Nevertheless, differences between treatments (significant to prove any effect) were not determined. Applied UVA radiation did not significantly inhibit the growth of any of the three algae and thus did not "simulate" a photocatalytic effect.

Because photocatalysis was effective against *Stichococcus* sp. in an earlier study (Gladis et al., 2010), this isolate was chosen as the test organism to investigate cellular viability. Despite growth inhibition on photocatalytically active filters (Gladis et al., 2010), the viability of algal cells was not impacted on glass shards. Permeable (destroyed) membranes were found in <2% of all cells (Fig. 5). Photocatalytically coated shards did not trigger oxidative stress in algae, as >90% of the cells were viable. Esterases were active in 95% of the cells. Thus, no photocatalytic effect was measured even after 48 h of irradiation with UVA.

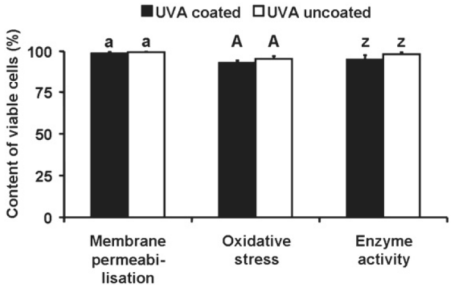

Fig. 5. Portion of viable cells (%) of *Stichococcus* sp. (SAG 2060) detected as membrane integrity, oxidative stress and esterase activity after 48 h incubation under UVA on photocatalytically coated and uncoated shards. Shown are means +/− SD ($n = 3$). Letters represent the absence of significant differences ($p \leq 0.05$).

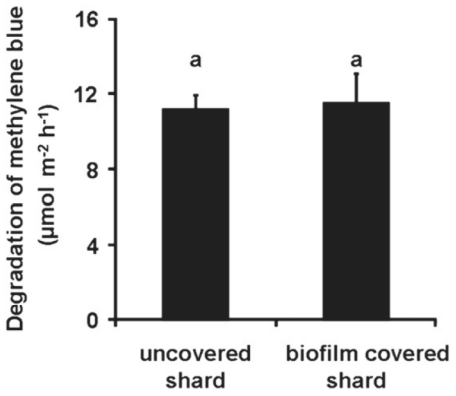

Fig. 6. Degradation of methylene blue ($\mu mol\ m^{-2}\ h^{-1}$) on uncovered shards and biofilm-covered photocatalytically coated shards under $4\ W\ m^{-2}$ UVA. Shown are means +/− SD ($n = 6$). Letters represent significant differences ($p \leq 0.05$).

3.4. Influence of algal biofilms on chemical activity

Photocatalytically coated shards degraded methylene blue at a rate of 11 $nmol\ m^{-2}\ h^{-1}$ (Fig. 6). Although the biofilms of *"Chlorella" luteoviridis* visibly stained the shards, methylene-blue degradation did not decrease. The biofilm-covered area was 12% on average. Compared to the algal biomass on the shards used in the photocatalytic-effects investigation (see Section 3.3), this was rather high. At the end of the incubation, 1.7% of shard area (on average) was covered by *"Chlorella" luteoviridis* and *Coccomyxa* sp. That biomass corresponded to $0.09 * 10^6$ cells cm^{-2} for *"Chlorella" luteoviridis* and $0.18 * 10^6$ cells cm^{-2} for *Coccomyxa* sp. *Stichococcus* sp. covered an average of 1.4% of the area of shards (corresponding to $0.09 * 10^6$ cells cm^{-2}).

4. Discussion

4.1. Potential of photocatalysis for algal growth prevention

Investigated photocatalytic surfaces did not reduce aeroterrestrial algal growth, although their ability to degrade methylene blue was high. This implies that chemical activity is not directly comparable to biological activity. While only minor modifications are needed to alter the structure and decolourise the dye, inactivation of microorganisms is much more complex and involves many more radicals (Marugan et al., 2008). Photocatalysis affects microorganisms by the oxidation of cell walls and membranes (Kiwi and Nadtochenko, 2005). Therefore, resistance of organisms, such as bacteria and yeast, increases with cell wall complexity (Kühn et al., 2003). Because algal cell walls are generally thicker than bacterial cell walls, photocatalysis against algae is less effective than against bacteria. In addition, aeroterrestrial algae are protected (e.g., by EPS) and can cope with oxidative stress (Mallick and Mohn, 2000). In algae, oxygen radicals are always formed during electron transport in photosystems. Thus, algae are protected by enzymatic and non-enzymatic defence mechanisms (e.g., superoxide dismutase, tocopherols and carotenoids; Mallick and Mohn, 2000). Photocatalytically generated radicals that enter cells could also be detoxified by these mechanisms.

The alga *Stichococcus* sp. did not grow stably on glass shards and thus the photocatalytic effects on its growth could not be assessed. Cell-specific viability of *Stichococcus* sp. could be evaluated but did not show any reaction to photocatalysis on glass shards (Fig. 5). This alga was affected by photocatalysis under conditions of higher water availability (Gladis et al., 2010). Gladis et al. (2010) investigated TiO_2 particles that were not fixed in material and that were potentially transported into algal cells; therefore, they affected cells more efficiently than did photocatalytic coatings (Kahru et al., 2008). Additionally, in that study, algae grew on filters where the formation of real EPS-enveloped biofilms should be reduced because algae are better supplied with water than on shards and protective structures are less necessary. Perhaps growth under conditions of desiccation stress on the shards in our study promoted adaptations to adverse conditions and increased algal resistance. Protection mechanisms of algae, bacteria and fungi should be investigated in more detail to assess photocatalysis as a disinfection measure. Efficient disinfection must be questioned, because many bacteria develop spores with protected cell walls. Reduced photocatalytic effects against inactivate bacterial and fungal spores have already been observed (Vohra et al., 2005; Sichel et al., 2007).

Photocatalytic activity has been shown to be improved by increased UVA intensities and higher TiO_2 concentrations (Benabbou et al., 2007). Modification of TiO_2 and other photocatalysts also improved activity and made the photocatalyst sensitive to visible light (Hashimoto et al., 2005). So far, photocatalytic effects on aeroterrestrial algae are still scarcely investigated and should not be generally denied under natural conditions.

In contrast to modified photocatalysts, UVA radiation in natural habitats cannot be increased and should not be raised above natural levels in efficiency tests. Moreover, photocatalysis needs to be effective even under very low UVA. If it is not, biofilms may develop during periods or in places with lower UVA. Even under high UVA, biofilms can succeed because they shade themselves and the (photocatalytic) material with layers of "sacrificed" cells. Additionally, higher UVA in tests may inhibit growth in controls as was observed in this study at $7\ W\ m^{-2}$ UVA.

4.2. Testing photocatalytic efficiency

This study thoroughly investigated the efficiency of photocatalysis to prevent algal growth on building materials. Although its results did not find any effect of TiO_2-coated glass on algal viability, this was not due to methodical deficiencies but rather to the characteristics of aeroterrestrial algae (see Section 4.1.). Hence, this methodology is suggested as a standard for investigating other photocatalytic materials with (likely) higher photocatalytic activity. Some important facts must be considered for tests of anti-algal surfaces, as efficiency tests for chemicals and established methods for bacterial inactivation by photocatalysis are not transferable to algal incubations. Most studies on inactivation of microorganisms by photocatalysis used colony-forming units (CFU) as viability parameters (e.g., Benabbou et al., 2007; Sichel et al., 2007). However, CFU counting detects only rapid growth of highly viable cells (Leff and Leff, 1996; Nakajima et al., 2005) and thus cannot be recommended for efficiency tests or surviving cells. Sub-lethal damages that inhibit rapid growth may remain unrecognised (false negative) or subsequent recovery of microorganisms may occur (false positive). In this study, growth was chosen as the parameter to detect photocatalytic activity because it is sensitive and it integrates different types of damages including sub-lethal effects.

Nevertheless, growth represents the average response of the population. Thus, cell-specific parameters were also measured to provide the number of affected and unaffected cells and thus the probability of recovery. This differentiation is very important for predicting biofilm growth on man-made surfaces, especially over the long-term (Gladis et al., 2010). The combination of both average growth and cell-specific parameters results in a reliable detection

of effects on both the cell and population (or community = biofilm) levels.

Photocatalysis is activated by radiation. Thus, the biomass of algae in the tests must be thin and single-layered to minimise surface shading and self-shading by algae, which would lead to various differently adapted and active cells. Such biomass is too low to be measured with established methods (e.g., the sensitive PAM fluorometry) (Eggert et al., 2006). Fluorescence microscopy combined with image analysis was well suited to detect algal biomass of such thin biofilms. Although only small portions (i.e., areas of 1.0–1.5 mm^2) of the test specimens were screened, biomass increase was reliably measured on controls. Nevertheless, biofilms are highly heterogeneous. Therefore, the detection of growth on surfaces must be conducted on at least 20 images for each data point and on three test specimens as replicates.

Numerous studies on photocatalytic activity use simple controls without UVA or photocatalysts (e.g., Sunada et al., 2003; Benabbou et al., 2007). Undoubtedly, only comparison with controls allows for the assessment of the photocatalysis. Nevertheless, the mechanism of photocatalysis is activated by UVA and requires more than one control treatment (with and without UVA and with and without coating). Otherwise, photocatalytic effects may be masked by the effects of UVA or of photocatalysts (Hund-Rinke and Simon, 2006). The present study investigated three different control treatments to separate any effects of UVA or of coating by photocatalysis (Fig. 1). In this way, the influence of UVA treatments could be separated. The control without TiO_2 and UVA detected no coating effects on shards, although the material's wettability was obviously hydrophobic in contrast to superhydrophilic photocatalytically active surfaces. Daily sprinkling supplied algae with optimal amounts of water and direct coating effects were reduced. Although photocatalysis consists of oxidation and wettability effects, the development of the presented test design was focussed on radical formation. The combination of both effects should be detected in long-term examinations (e.g., outdoor weathering). However, algal biofilms were thus far not suppressed by photocatalytic coatings under conditions of outdoor weathering (Gladis and Schumann, 2011).

Another aim of this study was to obtain controls by simulating natural conditions on buildings (which also favour growth). Habitats of aeroterrestrial algae are characterised by low UVA radiation, as they typically colonise shaded building sites where evaporation and radiation stress are reduced. Thus, the applied UVA intensity was low when compared to other studies but was sufficient as shown by the degradation of methylene blue (Fig. 6). This may explain why other studies found photocatalytic effects even on algae (e.g., Frach et al., 2007).

In addition, aquatic algae, which are often used as the model species, are more susceptible to UVA because it is readily attenuated by water bodies and plankton communities. In contrast, aeroterrestrial algae are well adapted to UVA (Karsten et al., 2007a) and the applied radiation did not affect the algae here used. These organisms are adapted to life at the interface between the atmosphere and a solid surface and thus also to low water availability. To survive adverse conditions, they develop thick cell walls and excrete EPS (Karsten et al., 2007b). Therefore, the right target organisms, i.e. aeroterrestrial algae, need to be determined.

Not all aeroterrestrial algae grow on surfaces under laboratory conditions. For instance, *Apatococcus* spp. dominate many natural green biofilms but are difficult to culture (Gustavs, pers. comm.). Their growth was too slow to be separated from any inhibiting effect. Thus, *Apatococcus* isolates will not be suitable as test organisms until their optimal culture conditions are discovered. However, the high prevalence of *Apatococcus* spp. in nature indicates perfect adaptation and many good defence mechanisms against stress. Therefore, results with aquatic model species or less-resistant terrestrial strains may not be easily extrapolated to natural biofilms (as with *Apatococcus* spp.). The use of dominant, as well as diverse, organisms in anti-algal tests will test the different adaptation strategies of algae and provide a reliable assessment of growth prevention.

Within liquid culture, aeroterrestrial algae are supplied with water and nutrients and defence adaptations may be reduced. Subsequently, algae may lose resistance against both environmental stresses (e.g., drought) and microbicidal attack (e.g., photocatalysis). Thus, photocatalytic efficiency must be tested under natural conditions where adaptations are developed.

Outdoor weathering reflects natural conditions but is time-consuming and susceptible to disturbances (Gladis and Schumann, 2011). Thus, test conditions in the laboratory should be similar to natural conditions on surfaces and test specimens should be exposed to air. Because algal biofilms grew slowly or not at all under water stress (Häubner et al., 2006), a compromise between near-natural conditions and high water availability is needed. This study was conducted under low-light conditions (PAR and UVA), a Light/Dark-rhythm of 16/8 h, 100% air humidity, mild temperatures and daily sprinkling with diluted medium.

The presented test system was developed to assess the photocatalytic efficiency of building materials under near-natural conditions. Nevertheless, not all potentially influencing factors could be adjusted in laboratory. (1) The photocatalytic-disinfection process was observed to be sensitive to the presence of inorganic and organic matter (Marugan et al., 2010). (2) The effects of superhydrophilicity of photocatalytic surfaces should reduce adhesion of aeroterrestrial algae and reduce water availability by rapid drying (Messal, 2007). (3) Environmental stress will influence the resistance of target organisms (e.g., Guasch and Sabater, 1998). Nevertheless, the results of this study are transferable to natural conditions as they conclusively simulate these conditions in the laboratory by using multiple control sets.

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3.3 Influence of material properties and photocatalysis on phototrophic growth in multi-year weathering

Im Freiland sind aeroterrestrische Algen durch ihre Anpassungen an ihr extremes Habitat, welche sie unter Kulturbedingungen verlieren können, vor ungünstigen Bedingungen geschützt. Im Gegensatz zu Laboruntersuchungen sind die Bedingungen hier nicht optimiert, sondern realistisch und „echt". In dieser Arbeit wurde der Einfluss verschiedener Dachziegelmaterialien auf die Entwicklung von natürlichen Algenbiofilmen im Freiland untersucht. Dem naturbelassenen Ton mit einer hohen offenen Porenweite standen engobierte und glasierte Materialien gegenüber. Daneben wurden auch photokatalytisch beschichtete Dachziegel untersucht. Die Dachziegel sind an sechs Standorten unterschiedlicher klimatischer Bedingungen in Deutschland über mehrere Jahre bewittert worden. In regelmäßigen Abständen wurde die Algenbiomasse auf den Materialien mittels Pulsamplitudenmodulations-(PAM-) Fluorometrie, Bildanalyse und visueller Bewertung quantifiziert.

Dachziegel aus naturbelassenem Ton waren am stärksten mit Algen bewachsen. Auf engobierten Ziegeln entwickelten sich die Algenbiofilme weniger intensiv. Dagegen waren schwarz glasierte Ziegel kaum vom Biofilmwachstum betroffen. Das beschriebene Bewuchsmuster deckte sich mit der gemessenen Wasserverfügbarkeit auf den Ziegeloberflächen. Während die offenporigen Materialien viel Wasser speicherten, mit dem die Algen sich versorgen konnten, trockneten die glasierten Ziegel schnell ab und hemmten dadurch das Algenwachstum. Wasserverfügbarkeit war der Schlüsselfaktor für das Wachstum von Algenbiofilmen im Freiland. Durch die entsprechende Anpassung der Materialeigenschaften lässt sich der Bewuchs durch Algen also unterdrücken, jedoch an Standorten mit ansonsten günstigen Bedingungen nicht vollständig verhindern. Zudem ermöglichten geschützte Strukturen, wie Überlappungszonen und Kanten, ein ungestörtes Wachstum von Algen auch auf ungünstigen Materialien. Photokatalytisch beschichtete Dachziegel waren in gleichem Maße vom Algenbewuchs betroffen wie unbeschichtete Materialien. Die Photokatalyse war also nicht gegen Algenwachstum wirksam. Damit bestätigte diese Arbeit die Ergebnisse der simulierten Biofilmbildung unter Laborbedingungen.

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Influence of material properties and photocatalysis on phototrophic growth in multi-year roof weathering

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ABSTRACT

Phototrophic growth on roofs leads to weathering and impacts their appearance. Roof tiles with various properties are available (natural clay, engobed, varnished or coated with photocatalytic TiO_2). The aim of this study was to examine the influence of materials on the development of phototrophic biofilms. Roof tiles were weathered in six climatic regions in Germany for several years. Phototrophic biomass was periodically determined by PAM-fluorometry, image analysis, and visual evaluation. Roof tiles of natural clay were the most heavily infested, while black varnished roof tiles were hardly covered with any phototrophs. This colonisation pattern was compared to water availability on roof tiles surfaces. In contrast to rough natural clay, varnished black tiles accumulated less water, dried quickly, and were rather resistant to phototrophs. The photocatalytic coating was not effective against phototrophic growth. Materials with appropriate properties may prevent phototrophic growth without biocides through reduced water absorption capacities and by avoiding radiation protected structures.

1. Introduction

Weathering of roof tiles leads to soiling of material, changes in solar reflectance, and physical disintegration (Berdahl et al., 2008). Degradation may be caused by abiotic factors, such as wind, sunlight, rain, hail, snow, atmospheric pollution, and temperature, but it can also be caused by the growth of biofilms, which are microbial cells embedded in extracellular polymeric substances (EPS) for adhesion and protection. The polymers can solubilise components of the building material and damage material by repeated wetting and drying cycles and subsequent expansion and contraction, as well as blocking evaporation (Scheerer et al., 2009, and references therein). Discoloration by pigments and dirt particles adhering to EPS is not only an aesthetic problem, but may absorb more sunlight, which increases physical stress (Scheerer et al., 2009 and references therein). Furthermore, bacteria and fungi excrete organic and inorganic acids, which alter the chemical structure of materials (Gómez-Alarcón et al., 1994; Gaylarde et al., 2001). In addition, filamentous microorganisms may penetrate the material and burst substance (Ortega-Calvo et al., 1991).

Green algae are frequent colonisers of buildings and dominate biofilms in Europe (Gaylarde and Gaylarde, 2005). They are present in air and rainwater (Brown et al., 1964; Schumann et al., 2004) and develop when conditions are suitable. At one of our stations (Zingst) succession and species composition in roof tile biofilms were investigated and *Apatococcus* was found to be the dominant genus (M. Görs, pers. comm.). Phototrophic biofilms are the base of other, more conspicuous or even harmful organisms, such as lichens, mosses, and fungi, which utilize products of phototrophic primary production for heterotrophic nutrition.

Biocides are widely used on building materials, such as plaster and paint, to prevent microbial growth. Organic biocides are not applicable for roofs since they degrade during the tile-firing procedure. Heavy metals may be effective against biofilms on roof tiles. However, their content in building material is strictly regulated (e.g., for Germany LAGA, 2003), since they readily run off from roofs and pollute soil and water (Hillenbrand et al., 2005). Established biofilms may be removed by, for example, high-pressure water blasters, but these removal mechanisms may also be aggressive against material surfaces. Thus, the prevention of damage by microorganisms is a more sustainable measure of surface protection.

The growth conditions on roof tiles are extreme. Phototrophs have to cope with high amplitudes of radiation and temperature, and changing water availability, which is the main growth-influencing factor for aeroterrestrial growth. Häubner et al. (2006) measured positive growth of phototrophs and optimum photosynthesis at 100% air humidity, while less humidity inhibited phototrophic viability. Mild climate and adjacent vegetation triggered phototrophic growth on buildings (Barberousse et al., 2006). Furthermore, the weather and the north sides of buildings (in the

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northern hemisphere) are more strongly infested by phototrophs (Barberousse et al., 2006). Aeroterrestrial phototrophs are well-adapted to survive periods of unsuitable conditions by, e.g., thick cell walls, EPS, and osmolytes (Karsten et al., 2007; Gustavs et al., 2009). Nevertheless, building materials may prevent phototrophic growth over the long term by decreasing water availability and providing poor adhesion properties (Barberousse et al., 2007a).

Manufacturers produce many kinds of roof tiles that differ in surface treatment, material, or colour. Engobed roof tiles have a reduced open pore volume at the surface and, therefore, a lower water absorption capacity. Glazed surfaces reduce water absorption and their hydrophobicity promotes dripping off of water drops. By contrast, photocatalytic coatings containing mostly titanium dioxide (TiO_2) are hydrophilic under UV irradiation, which leads to water spread and rinse off as closed water film (Fujishima et al., 2007). Additionally, photocatalytic surfaces form highly reactive hydroxyl radicals, which degrade organic molecules (Mills and LeHunte, 1997) and probably affect microbial colonisers. Although such self-cleaning coatings are already applied on tiles, their potential to prevent biofouling on roofs is not yet tested.

Although there has been research into factors influencing phototrophic growth, detailed data about weathering on artificial surfaces by phototrophs are scarce. Long-term data about the effectiveness of photocatalytic coatings are completely lacking. Thus, the aim of this study was to examine the influence of different materials on phototrophs over the long term. To do this, roof tiles differing in origin, surface material, and coating were weathered at six geographically different sites with temperate climate. Phototrophic biomass and biofilm expansion was determined regularly by PAM-fluorometry, image analysis, and visual evaluation to support the hypothesis that water availability is the main factor influencing weathering by phototrophs and to evaluate photocatalytic effectiveness against phototrophic growth.

2. Materials and methods

2.1. Roof tiles and study sites

Roof tiles were exposed at six locations, which were spread over Germany and covered different temperate conditions (Table 1). Detailed meteorological data on the sites will be presented elsewhere within a climatic context. Racks with roof tiles were set up in autumn of 2002 and examined until April 2006. An exception was the northernmost station, Zingst, which was set up half a year later and studied until April 2009. Admittedly, the winter period of 2002/2003 was missed, but since the conditions for phototrophic growth are adverse in this season, the consequences for weathering were considered as marginal. The racks were positioned at an angle of 45° in the northeast direction. Roof tiles were set up 5–7 cm apart to avoid rainwater rinse off from the upper tiles onto lower ones. Each rack consisted of 18 clay roof tiles, which varied in origin, surface treatment, and coating (Table 2) and were produced by Dachziegelwerke Nelskamp GmbH,

Table 1
Stations of roof tile racks with geographic coordinates and dates of set up and last examination.

Station	Geographic coordinates		Date of set up	Date of last examination
	Latitude	Longitude		
Zingst	54°26′25"	12°41′22"	April 2003	September 2009
Schönerlinde	52°37′34"	13°25′53"	December 2002	April 2006
Schermbeck	51°41′35"	6°52′20"	November 2002	April 2006
Göttingen	51°33′32"	9°57′17"	December 2002	April 2006
Unsleben	50°22′35"	10°15′37"	December 2002	April 2006
Ostrach	47°57′45"	9°14′23"	October 2002	April 2006

Table 2
Specimen numbers, origin, surface material and presence of photocatalytic coating of roof tiles.

Origin	Tile number					
Factory 1: Schermbeck	1	2	3	4	5	6
Factory 2: Unsleben	7	8	9	10	11	12
Factory 3: Groβ Ammensleben	13	14	15	16	17	18
Surface material	Red engobe		Natural clay		Black varnish	
Photocatalytic coating	+	−	+	−	+	−

Germany. A set of six tiles were produced in three different factories to assess if different raw materials influenced biofilm formation. In each case two of these six tiles had a surface of red engobe. Engobes are surface refinements of clay with a smaller pore size than that of pure clay. While two other tiles had a natural surface of pure red clay, the last two tiles were glazed with a black varnish. Varnishes are hard covers, which seal the surface. In each case one of the two similar tiles per set was coated by photocatalytic TiO_2. The photocatalytic coating with 4–5 mg cm^{-2} TiO_2 was applied by sol-gel technology, which is used to prepare functional coatings (Aegerter et al., 2008). To evaluate the impact of an overlapping array, as is normal for roofs, eight roof tiles were imbricated at the Zingst station in late 2004, numbered 19–26, and examined as the other roof tiles.

Phototrophic biomass was examined at least once per year within one week at all sites. The racks were examined for the first time in August 2003; this was followed by surveys in April and December 2004, and April 2005 and 2006. The Zingst station was examined additionally in October 2006, May 2007, September 2007, September 2008, and April 2009.

2.2. In-vivo chlorophyll a fluorescence

A PAM-2000 fluorometer (Heinz Walz GmbH, Germany) was used to determine the *in-vivo* chlorophyll (chl) *a* fluorescence (F_t) as an indicator of biomass. Eggert et al. (2006) have described the measurement of the initial chlorophyll fluorescence (F_0) as a specific biomarker. As we found a strong correlation between F_0 in the dark (night) and F_t under ambient light (day, $R_S = 0.8$, $p < 0.001$), outdoor measurement of F_t without dark adaption was performed. Measurements were performed in the early morning or late afternoon to exclude phototrophic performance depression, which occurs often around noon. A 0.6-s far-red pulse (735 nm, intensity setting 7, approx. 180 μmol photons m^{-2} s^{-1}) fully oxidised electron transport chain prior to measurement. F_t was recorded with 600 Hz pulsed red measuring light (650 nm). The measuring light intensity of the instrument was 10 and the gain 3 in the first examinations (August 2003, April 2004, and December 2004). The optical fibre was always placed at a right angle 12 mm above the sample. To optimise the detection of low phototrophic biomass, settings were improved, from the fourth examination in April 2005 on, to a light intensity of 7, a gain of 6, and a distance of 7 mm. Data of measurements with both settings on the same day were just as variable than spatial variability, since the coefficient of variation was calculated to be 50% respectively 44% on average. Hence, data were plotted together. Ten minutes after moistening with tap water, three positions on each tile were measured with the PAM-2000 – one at the bottom, the centre, and the top (Fig. 1A). To compare material properties, the highest value of these three measurements, referred to as $F_{t\ max}$, was determined.

2.3. Image analysis of photographs

All tiles were photographed at a resolution of four megapixels with a Nikon Coolpix 995 camera. One pixel had a size of about

Fig. 1. Photographs of roof tile number 16 at the station Zingst (weathering since April 2003). A) August 2003, B) April 2004, C) December 2004, D) April 2005, E) April 2006, F) October 2006, G) May 2007, H) September 2007, I) October 2008, J) April 2009, K) September 2009. Circles in A show the three areas for *in-vivo* chl *a* fluorescence measurement and visual evaluation: bottom, centre and top.

0.2 × 0.2 mm. The extent of green and black coloured areas was quantified with the Software AnalySIS Pro 3.02 (Olympus Soft-Imaging GmbH). Since illumination and colour of all the materials and at the surveys differed, the threshold values for RGB channels were adjusted manually for each photograph. The amount of phototrophic-covered area was calculated in relation to the whole tile area. This total area was measured as total pixels within a manually defined region of interest (ROI), which also excluded background areas from the biofilm measurement.

2.4. Visual evaluation

The intensity and coverage of biofilms were differentiated into four classes, which were backed up by chl *a* quantification (Schumann et al., 2005). Again, three areas were evaluated on each tile: bottom, centre, and top (Fig. 1A). Areas without any visual phototrophic growth were assigned zero points. One point was given for light discoloration or scattered phototrophic growth. Obvious covering at several spots or widespread light green growth was assigned two points, while obvious and widespread dark discolorations received three points. The number of points from all three subareas per tile were summed up (maximum points per evaluation = 9).

2.5. Abiotic parameters on the roof tiles

The surface moisture and temperature of tiles were measured at the northern station, Zingst, to characterise all different materials. Available water was estimated by absorption into 4-cm^2 pieces of

nappy (Pampers BabyDry, Procter & Gamble, Germany) according to Häubner et al. (2006). Therefore, nappy was cutted in their central area and packed in thin filter paper to avoid a loss of absorbing material. The pieces were manually pressed onto the tile for 10 s. Four replicates were analysed for roof tiles 1–6. The temperature of all tiles was measured at three points per tile by an infrared thermometer (PCE-888, PCE Deutschland GmbH, Germany) in the morning to estimate thermal conductivity.

2.6. Statistical analyses

The dispersal and thickness of biofilms was not normally distributed. Significant differences in data were analysed with the non-parametric Kruskal–Wallis test followed by the pairwise difference Mann–Whitney test and significance level test after Bonferoni. Spearman rank order analysis was used to identify correlations between F_0 and F_t. The statistical reports were done with SPSS software (version 15.0).

3. Results

3.1. Abiotic growth conditions on the roof tiles

Photocatalytically coated and uncoated tiles exhibited only minor differences in surface moisture (3.7 versus 2.2 ml m^{-2}, respectively; $p = 0.38$) and temperature (12.2 and 2.9 °C, respectively; $p = 0.12$). The comparison of uncoated materials detected high amounts of available water on natural clay tiles (4.2 ml m^{-2}) and rather low surface temperatures (12 °C, Fig. 2). Black varnished roof tiles accumulated much less water and were almost 2 °C warmer than the other tiles. On roof tiles of red engobe, the level of water availability was medium (2.1 ml m^{-2}) and temperatures were similar to that of natural clay tiles.

3.2. Phototrophic growth

Phototrophs developed within one and a half years into a thin but widespread biofilm on the sample roof tile shown, number 16 in Zingst, an uncoated tile of natural clay (Fig. 1). The green biofilm was at its most extensive in October 2006 and decreased again later, but did not disappear completely. Therefore, visual examination scored 9 points each time between April 2006 and September 2008 (Fig. 3). *In-vivo* chl *a* fluorescence detected the first phototrophic biofilm with F_t values above detection level (0.03 V) after two years. Highest F_t values >0.19 V for all three areas were measured at the maximum of growth. Values for F_t decreased within the next three years back to detection level.

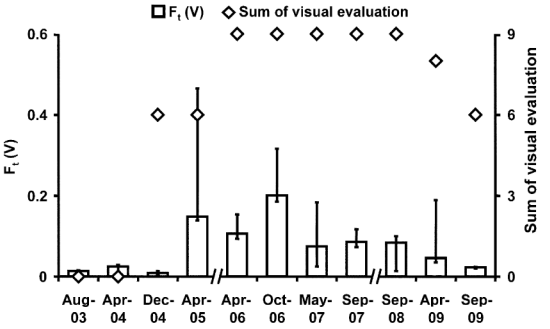

Fig. 3. *In-vivo* chl *a* fluorescence F_t (V) and visual evaluation score on roof tile number 16 at the station Zingst during the examination period from August 2003 to September 2009. Shown are bars with median, minimum and maximum of F_t measurements ($n = 3$).

For most other uncoated roof tiles at all stations, growth was detected by *in-vivo* chl *a* fluorescence measurements earlier than by visual evaluation (Fig. 4). First growth was detected fluorometrically after 1.5 years on 50% of the tiles, while it took a minimum of 2 years for growth to become visible.

Average F_t values of all roof tiles at the Zingst station show a pattern similar to that of the sample tile, 16 (Fig. 5). First growth was found at the bottom of the tiles and spread from there during the next months. Later, no differences between tile areas were detectable. Although very high values of F_t were measured, e.g., in October 2006, the 25th percentiles were low because some roof tiles remained uncovered. *In-vivo* chl *a* fluorescence decreased from October 2006 to below detection level in September 2009 for two thirds of the measurements. The period of initial growth is most interesting for material assessment and, most often, the first 3 years are observed for material weathering. Therefore, the vegetation periods of April 2005 and 2006, when phototrophic growth was promoted by low radiation and high precipitation, were chosen as periods of interest to further compare roof tile weathering at different stations.

3.3. Phototrophic growth on different materials

The only station without any biofilm formation was Schönerlinde in northeast Germany. There must be causes for this lack of

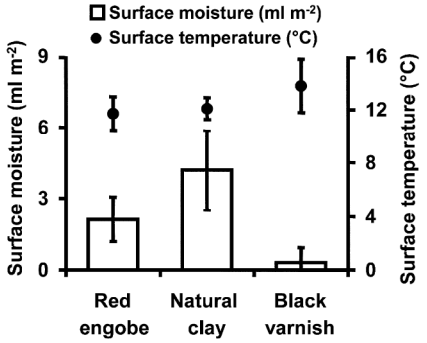

Fig. 2. Surface moisture (ml m^{-2}) and surface temperature (°C) of roof tiles without coating at the station Zingst in October 2009 differentiated into surface treatments. Means ± SD, $n = 4$ for surface moisture and $n = 9$ for surface temperature. Letters represent significant differences between the materials ($p \leq 0.05$).

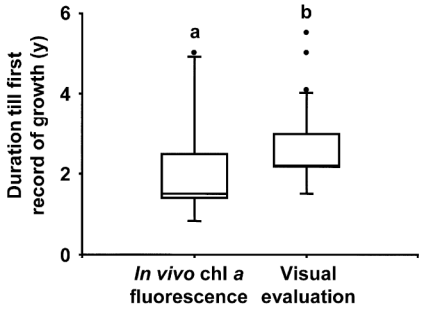

Fig. 4. Duration till first record of growth (years) by using *in-vivo* chl *a* fluorescence and visual evaluation to detect phototrophic growth on roof tiles without coating at five stations in April 2005 and April 2006. Shown are 25th and 75th percentiles as boxes with median as line, 10th and 90th percentiles as error bars and dots as outliers ($n = 90$). Letters represent significant differences between the materials ($p < 0.05$).

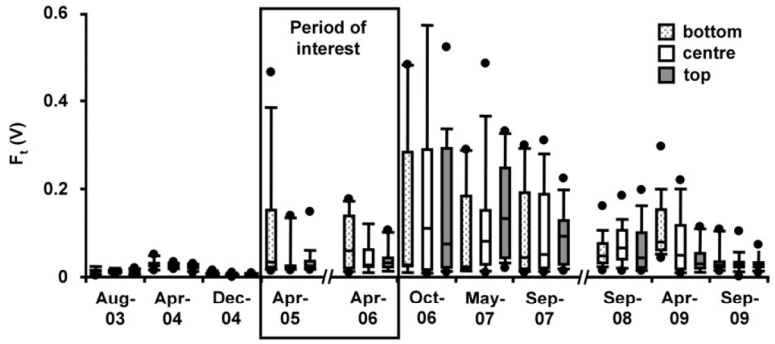

Fig. 5. *In-vivo* chl *a* fluorescence F_t (V) at bottom, centre and top of all roof tiles at the station Zingst for each examination between August 2003 till September 2009. Shown are 25th and 75th percentiles as boxes with median as line, 10th and 90th percentiles as error bars and dots as outliers ($n = 18$). The frame marks the period of interest that was analysed at all stations.

infestation other than material properties, since the same specimen were exposed. These results were not evaluated in this study. Nevertheless, this phenomenon will be discussed elsewhere within a climatic context.

Different originating factories (and with that raw materials) did not influence $F_{t\ max}$ on roof tiles without any self-cleaning coating. Their medians lay between 0.11 and 0.17 V (Fig. 6A). The covered area corresponded well with $F_{t\ max}$ (Fig. 6B) and also did not differ between material origins. Photocatalytically coated roof tiles did not differ in raw material as well (Fig. 7A and B). Consequently, roof tiles from different factories were regarded as replicates in the following.

In contrast, the kind of surface treatment used had strong effects on phototrophic growth. Tiles with black varnish were hardly supported growth and median of $F_{t\ max}$ values were well below the detection level. All tiles of red engobe and natural clay had visible phototrophic growth (Fig. 8A). The median of $F_{t\ max}$ was 0.19 V for red engobe and 0.42 V for natural clay. Only 1 out of 30 black varnished roof tiles was covered >1% by phototrophs. Tiles of red engobe and natural clay were covered by up to 47% and 74%, respectively (Fig. 8B). Three quarters of the tiles with a natural clay surface were covered >1%.

The $F_{t\ max}$ of uncoated roof tiles was not significantly different from values of tiles with a photocatalytic coating (Fig. 9A). While the interquartile distances were rather similar, the median of coated tiles was even slightly higher. The biofilm cover on uncoated tiles was similar to that of coated tiles (Fig. 9B). The median of phototrophic-covered area was below 1% for both photocatalytic-coated and uncoated tiles and three quarters were covered up to 8% and 7%, respectively. The photocatalytic coating applied in this study did not prevent phototrophic growth.

By contrast, structural characteristics of roof tiles impacted phototrophic development. Thick biofilms appeared in cracks or at damaged spots, in shaded areas, and particularly at the lower edge of the tiles (Fig. 10A). Additionally, another study by the authors demonstrated that thick biofilms established below the overlap on the next tile, when tiles were imbricated as on normal roofs (Fig. 10B). Phototrophs may be spread further down by rain from there. Material properties did not markedly influence phototrophic infestation on these compromised zones (data not shown).

Phototrophic biofilms were also destroyed by deposited leaves and after strong rain, particularly in the centre. Many thick biofilms were grazed by snails as traces of their radula feeding were visible on the roof tiles (Fig. 10C). This seems to be an artefact as the tiles were exposed close to the earth. After dry and hot summers, especially in 2009, the F_t value was strongly reduced, although biofilms were still visible (Fig. 3).

4. Discussion

4.1. Investigation of phototrophic growth-preventing materials

Outdoor weathering over several years is the most common procedure used to estimate growth-preventing properties of roof tiles. Outdoor exposure ensures natural conditions, which are too complex to simulate in laboratory experiments. The main difficulties for climate simulation are high light, especially combined with lower temperatures, morning dew and inhibiting conditions, e.g., air

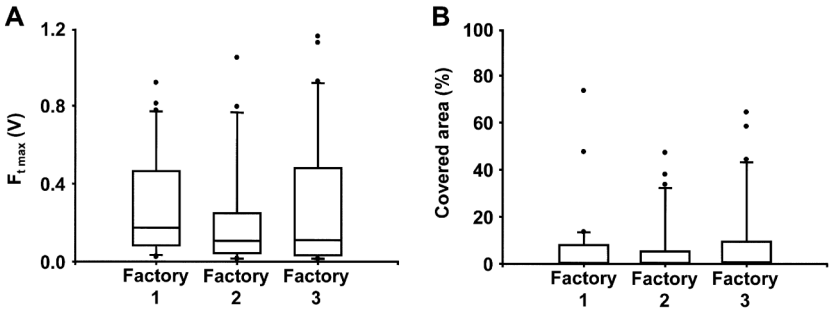

Fig. 6. A) Maximum *in-vivo* chl *a* fluorescence $F_{t\ max}$ (V) and B) covered area (%) of roof tiles without photocatalytic coating pooled from five stations in April 2005 and April 2006 differentiated into raw material origin. Shown are 25th and 75th percentiles as boxes with median as line, 10th and 90th percentiles as error bars and dots as outliers ($n = 30$).

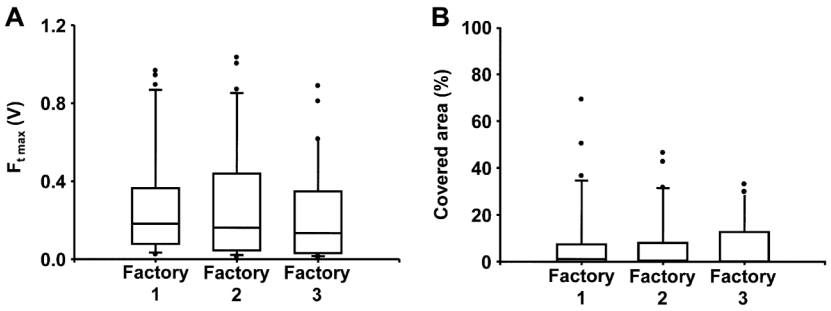

Fig. 7. A) Maximum *in-vivo* chl *a* fluorescence $F_{t\ max}$ (V) and B) covered area (%) of roof tiles with photocatalytic coating pooled from five stations in April 2005 and April 2006 differentiated into raw material origin. Shown are 25th and 75th percentiles as boxes with median as line, 10th and 90th percentiles as error bars and dots as outliers ($n = 30$).

pollution. Furthermore, outdoor incubation with natural soiling pressure reflects the complex process of colonisation, which includes competition and synergy between species. As a consequence, results of outdoor weathering are rather close to real material applications. However, outdoor experiments are time-consuming, and several important facts have to be considered to get transferable results. Natural weathering depends on the specific microclimate and other environmental conditions, e.g., whether the area is rural or industrial (Deflorian et al., 2008). Thus, a single station may lead to biased results. Especially unfavourable conditions or low infection pressure prevent phototrophic infestation, which has to be checked by so-called positive controls. These controls can be materials, which usually promote biofilms.

In this study, weathering stands were set up at six stations and incubated over a period of several years to consider local (micro) climates, and interannual and weather differences, e.g., cold winters or hot summers. Seasonal differences were also taken into account. One station was completely free of phototrophic activity, which may lead to misinterpretations due to a lack of positive controls. Also the remaining results were highly variable, although all weathering stands were within a geographically relatively small area (one country) and within the same climate zone (temperate). The investigation of several sites ensures that the results are transferable to other climate regions with similar colonising organisms, e.g., from Germany to France, but not to other major climate zones (Gaylarde and Gaylarde, 2005). It can be expected that aeroterrestrial organisms, such as cyanobacteria, which are dominant in tropical regions, are more resistant to high temperatures and dehydration due to their thick, pigmented sheaths than are green algae, which dominate biofilms in European climates (Gaylarde and Gaylarde, 2005).

Phototrophic biomass on artificial surfaces is quantified with different methods. Phototrophic biofilms on building surfaces are quantified mostly by visual evaluation of the intensity of green or black stains, where the colour is caused by phototroph pigments (e.g., Donner et al., 2002). Such semi-quantitative methods are accompanied by several problems. Apart from the subjectivity factor, illumination, reflection, material colour, moistness of the material, coincident covering with dirt or fungi, and distance to the object influence the impression. The image analysis of photographs can compensate for some of these problems and estimates the covered area reliably (e.g., De Muynck et al., 2009). Photograph analysis was appropriate especially in the upper measurement range (distinct biofilms) since it integrates the whole tile instead of only selected or prominent areas. Nevertheless, image analysis did not differentiate thin and thick biofilms. The colour of biofilms is not equal to biomass, since cellular pigment contents are species-specific (Jørgensen et al., 1991) and depend on accessory pigments, viability of phototrophs, and abiotic factors, such as solar radiation (Geider, 1987) and nutrient supply (Foy, 1993). The extraction of chl *a* as a phototrophic biomarker is well-suited to quantify phototrophic biomass on building materials (Schumann et al., 2005). This method is objective, allows replicates to estimate patchiness, and measures biomass of living phototrophs by excluding bleached cells. However, this extraction procedure is an invasive measure, at least for biofilms, so that successions and growth cannot be investigated at the same spot. Eggert et al. (2006) developed a non-invasive method to quantify phototrophic biomass by PAM-fluorometry, which was also used in this study. The measured chlorophyll parameters correlated well with chl *a* analysis, especially in the low measurement range. This easy in-situ method evaluates phototrophic colonisation, especially

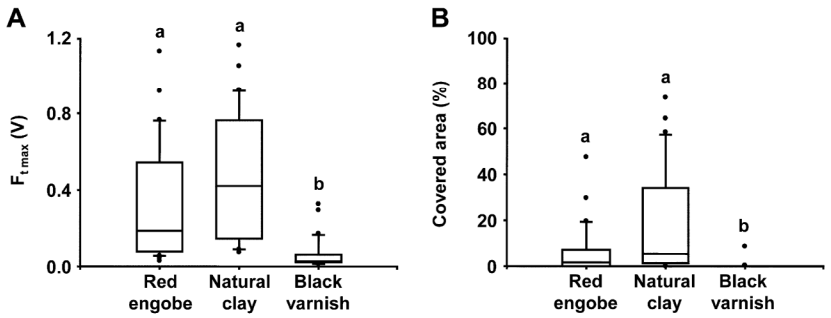

Fig. 8. A) Maximum *in-vivo* chl *a* fluorescence $F_{t\ max}$ (V) and B) covered area (%) of roof tiles without coating pooled from five stations in April 2005 and April 2006 differentiated into surface treatments. Shown are 25th and 75th percentiles as boxes with median as line, 10th and 90th percentiles as error bars and dots as outliers ($n = 30$). Letters represent significant differences between the materials ($p < 0.05$).

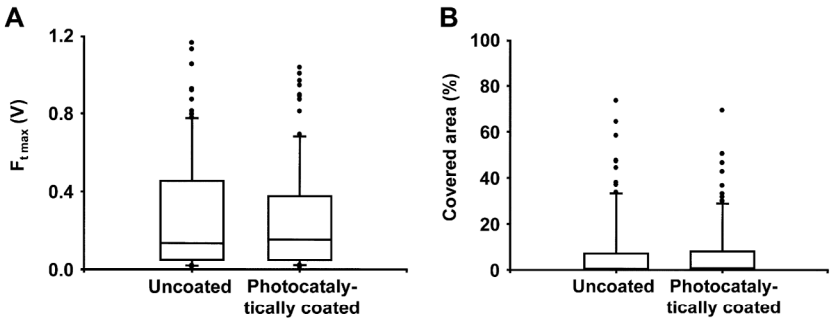

Fig. 9. A) Maximum *in-vivo* chl *a* fluorescence $F_{t\ max}$ (V) and B) covered area (%) of all roof tiles pooled from five stations in April 2005 and April 2006 differentiated into the presence/absence of photocatalytic coating. Shown are 25th and 75th percentiles as boxes with median as line, 10th and 90th percentiles as error bars and dots as outliers ($n = 90$).

initial growth. Additionally, information about phototrophic viability can be estimated (Häubner et al., 2006). Fluorescence measurements generally had a lower detection level compared to visual evaluation, since PAM-fluorometry detected phototrophic growth, which was not yet visible. In contrast, weakened or dead biofilms did not fluoresce but were visible. This occurred often after long-term desiccation stress in summer. A combination of fluorescence measurement and one visual method, image analysis or visual evaluation, is required for reliable estimation of phototrophic biofilms, because such a combination compensates for most specific weaknesses of the available methods.

4.2. Water availability as a key factor for phototrophic growth

Factors influencing phototrophic growth were separated into physical, chemical, and structural material properties. Physical properties include porosity, roughness, and temperature of the surface. Several studies found preferences by phototrophs for porous materials (e.g., Ortega-Calvo et al., 1995; Barberousse et al., 2007b). This study also found a strong impact of surface properties on phototrophic growth, which correlated well with open porosity at the surface. The major underlying mechanism is most likely a higher water absorption capacity of porous material, which was not measured directly, but is reflected in the surface moisture measurement. While natural clay tiles, which were grown upon most intensively, accumulated high amounts of water, the predominantly dry surface of the black varnished tiles did not support phototrophs (Figs. 2 and 8). Besides water availability, rough and porous surfaces, such as non-varnished tiles, favour adhesion of phototrophs (Barberousse et al., 2007b). Additionally, temperature on the black tiles was elevated, which may stress phototrophs on warm days. The heat storage capacity of black roofs can increase the temperature of the tiles up to 50 °C warmer than the ambient air (Berdahl et al., 2008). On a non-porous surface like black varnished tiles, morning dew will last as a thin water film, but due to their rapid warming, these tiles will dry faster than others. By contrast, surfaces of red engobe and, especially, natural clay, had large pores, which are supposed to accumulate morning dew and rain also below the irradiated surface, supply phototrophs with water. Additionally, Viles (2005) detected lower temperature amplitudes on wet surfaces, which stress phototrophs less. The consequences of the discussed physical properties are that surfaces with a low water storage capacity and high heat storage may prevent phototrophic growth on roofs.

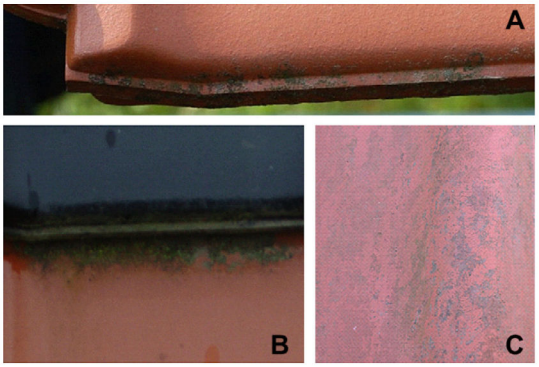

Fig. 10. Photographs of A) growth-promoting structures on roof tiles at the lower edge (roof tile number 8 at station Göttingen in April 2005) and B) the overlapping area (roof tile number 24 and 26 at station Zingst in October 2006). C) Photograph of partial destruction of phototrophic growth by snails (roof tile number 10 at station Zingst in September 2009).

The chemical composition of biofilm substrates may support phototrophic growth through nutrient supply or inhibit it by high pH or leaching of metals (e.g., mercury). Different raw materials differ in chemical composition, which may impact phototrophic colonisation (Ortega-Calvo et al., 1995). However, this study did not detect any correlation between raw material and phototrophic growth. This may be due to strict regulation of the heavy metal content of materials and products (e.g., for Germany, LAGA, 2003). Additionally, differences in substrate chemistry of raw materials were not analysed here. Scheerer et al. (2009) also found a low level of relevance of the chemical composition for stone colonisation. However, chemical characteristics are more important for coatings (Gaylarde and Morton, 1999).

Photocatalytic coatings are supposed to be effective against microbial colonisation. Other studies observed inhibition of phototrophic growth by such self-cleaning surfaces (e.g., *Chroococcus* sp.: Hong et al., 2005; *Cladophora* sp.: Peller et al., 2007). However, in this study, photocatalysis did not inhibit phototrophic growth at all. One cause may be insufficient UV radiation to activate photocatalysis. The roof tiles were positioned in the northeast direction, so that they were not exposed to direct sunlight or were exposed for a much shorter period. Therefore, shaded building sites are often affected most by phototrophic growth (Barberousse et al., 2006). In addition, these authors hypothesize that aeroterrestrial phototrophic cells are protected by thick cell walls and EPS against photocatalytically generated hydroxyl radicals. Furthermore, if phototrophs succeeded initially, e.g., on a more protected area or damaged surface, they may spread onto photocatalytic surfaces, because they inhibit UV

activation and/or may overgrow older, damaged cells or dirt. Thus, to predict effectiveness, the mechanisms of photocatalysis on phototrophs have to be clarified.

Phototrophic growth was not evenly distributed over the tiles, but rather concentrated in areas with structural impairments and on the bottom edge of tiles. Despite their growth-inhibiting properties, even black varnished roof tiles were visibly colonised, particularly at the lower edge of the tiles. The surface was rough here. Phototrophs growing in cracks were protected against water evaporation, radiation, and mechanical destruction by rain. Such structural features as edges and cracks increased biofilm heterogeneity and the diversity of colonising organisms (Murdock and Dodds, 2007). Such protected zones have to be avoided to prevent phototrophic growth. Nevertheless, production of a completely smooth surface may be costly, and imbricating always causes protected zones.

Phototrophic growth is not only controlled by properties of materials. Intensive phototrophic growth on several black varnished roof tiles indicated that phototrophs are still able to survive despite adverse material properties. Green biofilms are even visible on materials that do not accumulate any water, such as glass or Plexiglas (Brehm et al., 2005; Gladis, personal observations). Here, phototrophs use high humidity or rain. Whether these are special taxa remains to be investigated. On the other hand, some porous materials in this study did not sustain phototrophic growth despite their growth-promoting properties (station Schönerlinde). Häubner et al. (2006) described at least one species, which grew at 99% air humidity without liquid water. Thus, material properties may become secondary at specific climatic conditions. Further important factors on phototrophic colonisation of buildings were: (1) proximity of other objects, which can affect shading, wind turbulence, and propagation of germs; (2) internal building climate, which affects evaporation by means of temperature, thickness of walls, and insulation; and (3) micro-environmental influence, such as phototrophic grazers (Seaward, 1979).

5. Conclusion

This study confirmed water availability as a key factor in phototrophic colonisation of roofs and other building materials. Although phototrophs are well-adapted to desiccation, they do not resist such periods for long. The limits of water availability for aeroterrestrial phototrophs should be explored in more detail to predict algal growth under suitable conditions. Appropriate material properties may reduce or prevent phototrophic growth without biocides by controlling water absorption and heat storage capacities. Avoidance of growth-promoting structures can also prevent the development of phototrophic biofilms. In addition, climatic conditions may overlay material properties and enable phototrophic growth on well-protected materials. Photocatalytic surfaces did not affect phototrophic biofilms in outdoor weathering in contrast to our results from laboratory studies (Gladis et al., 2010). The causes for this discrepancy have to be investigated further. Therefore, the protective function of biofilms has to be clarified by investigating photocatalysis under nearly natural conditions.

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4 Diskussion

4.1 Physiologie aeroterrestrischer Algen

Die meisten Algen und Cyanobakterien leben in aquatischen Habitaten. Dennoch gingen beide Gruppen mehrmals zum Landleben über (Lewis & Lewis 2005, Cardon et al. 2008). Die Diversität phototropher Biofilme reicht von zahlreichen Gruppen der Grünalgen über Diatomeen bis hin zu Cyanobakterien, die sich vor allem in ihrer Pigmentzusammensetzung voneinander unterscheiden (Rindi & Guiry 2003, Rindi 2007). Um hemmende Effekte von Antialgenstrategien nachzuweisen, wird die Intensität des Algenbewuchses quantifiziert. Häufig wird die Biomasse des Algenbiofilms über das in allen Phototrophen vorkommende Chl *a* bestimmt. Der zellspezifische Gehalt ist jedoch stark art- und gruppenabhängig. Deshalb ist die Kenntnis der besiedelnden Organismen bei der Anwendung der Chl *a*- Fluoreszenz als Biomasseparameter von Bedeutung (z.B. Gladis & Schumann 2011a). Variationen der Größe der lichtsammelnden Antennen des Photosystem II, ihrer Anordnung bei Zellgrößenänderung sowie intrazellulärer Pigmentkonzentrationen führen außerdem zu veränderten Lichtabsorptionskoeffizienten, welche die Chlorophyllfluoreszenz der Algen beeinflussen (Krause & Weis 1991, Mitchell & Kiefer 1988). Auch ist der Einfluss von akzessorischen Pigmenten, z.B. der Phycobiline der Cyanobakterien, oder von anderen membranassoziierten Substanzen mit hohen Absorptionskoeffizienten auf die Güte dieser Methode noch nicht bekannt.

Während in den gemäßigten Breiten Mitteleuropas Grünalgen dominieren, überwiegen im subtropischen Klima Lateinamerikas Cyanobakterien (Gaylarde & Gaylarde 2005). Die Hüllen von aeroterrestrischen Cyanobakterien sind dicht und dunkel pigmentiert und bewirken eine hohe Resistenz gegenüber intensiver Sonneneinstrahlung (Büdel et al. 1997). Es gibt viele Hinweise, dass Cyanobakterien extrem widrige Bedingungen länger überleben können als Grünalgen (z.B. 25 Jahre Trocken- und Dunkelheit: Trainor 1985). Bei milden Temperaturen und höheren Niederschlägen scheinen sich jedoch eher die eukaryotischen Grünalgen, von denen die meisten zur Klasse der Trebouxiophyceae gehören, durchzusetzen (Rindi 2007, Mudimu 2008). Da in dieser Arbeit Algenisolate und Biofilme der gemäßigten Breiten untersucht wurden, liegt der Fokus der folgenden Ausführungen auf den hier dominanten aeroterrestrischen Grünalgen.

Die Anpassungen von Algen an die terrestrische Lebensweise werden in strukturellen und funktionellen Anpassungen unterschieden und beeinflussen die Substratbesiedlung sowie die Widerstandsfähigkeit gegenüber extremen Umweltbedingungen und Antialgenstrategien. Strukturelle Anpassungen umfassen alle morphologischen und biochemischen Eigenschaften der Zellen. Ein wesentliches Merkmal aeroterrestrischer Algen ist die morphologische Konvergenz (Rindi et al. 2009). Dies bedeutet, dass aufgrund von gleichen äußeren Bedingungen eine ähnliche Erscheinungsform in nicht verwandten Gruppen auftritt. Aeroterrestrische Algen entwickelten einen charakteristischen Morphotyp, mit dem sie sich an die Bedingungen in ihrem Habitat angepasst haben. Ihr Aussehen ist geprägt durch eine geringe Größe der einfach gebauten Zellen, die entweder einzeln vorliegen (*Coccomyxa, Stichococcus*), in kubisch angeordneten Kolonien (*Apatococcus*, Abb. 4.1 A) oder als Filamente (*Klebsormidium, Prasiola*) (Rindi 2007). Karsten et al. (2007a) vermuteten, dass sich die Algenzellen durch ihre dicken Zellwände an die trockenen Bedingungen im Habitat angepasst haben (Abb. 4.1 B). Grünalgen, auch aquatische, besitzen als Zellumgrenzung neben der Zellmembran eine Zellwand aus einem stabilen Cellulosegerüst. Im Gegensatz dazu haben z.B. Bakterien nur dünne Zellwände und sind mutmaßlich empfindlicher gegenüber äußeren Einflüssen (Madigan & Martinko 2009).

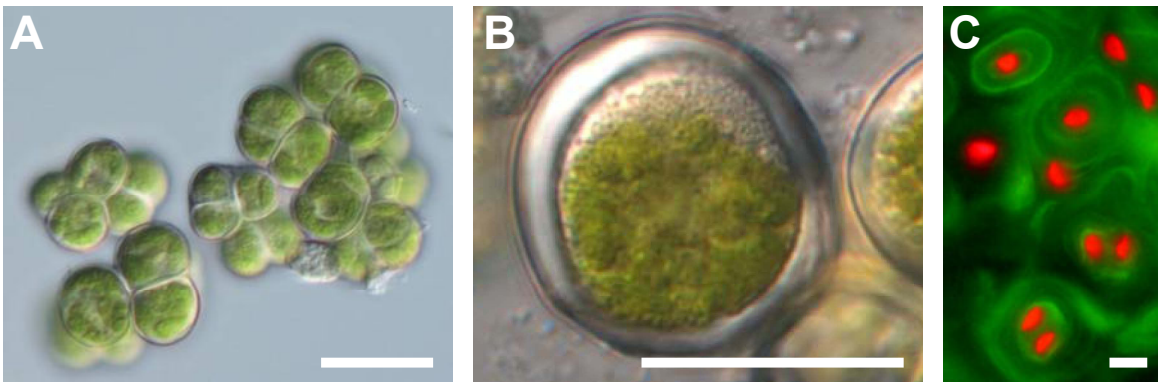

Abb. 4.1: A) Auch unter Kulturbedingungen wächst die in natürlichen Biofilmen dominante Alge *Apatococcus lobatus* (ROS 7/3) noch in Aggregaten. B) Dicke Zellwände einer *Apatococcus*-Zelle aus einem natürlichen Biofilm. C) Ein Fluoreszenzfarbstoff (ConA) macht die schützenden EPS-Hüllen der aeroterrestrischen Algen (unidentifizierte *Radiococcaceae* mit roter Chlorophyllfluoreszenz) sichtbar (Foto A: M. Görs, Fotos B und C: C. Hallmann, Maßstab 20 µm).

Eine wichtige Strategie der Algen in der Anpassung an ihr Habitat ist die Bildung einer schleimigen Schutzhülle aus EPS (Gorbushina 2007, Abb. 4.1 C). Da das Umhüllen mit EPS ein typisches Merkmal aller Biofilme ist, wird dieser Aspekt jedoch erst im folgenden Kapitel diskutiert.

Algen überdauern ungünstige Umweltbedingungen in dormanten Stadien, z.B. als Sporen (Agrawal 2009). Die Sporen der aeroterrestrische Grünalge *Chlorococcum* sp. konnten sogar noch nach 5jähriger Trocken- und Dunkelheit „wiederbelebt" werden (Klochkova et al. 2006). Sporen sind klein, leicht und von mehreren stabilen Schichten umhüllt. Ihre Funktion ist die Verbreitung der Organismen, z.B. über Wind, und die Resistenz gegenüber extremen Bedingungen, wie hohen Temperaturen, hoher Strahlung, Austrocknung und Nährstoffmangel (Madigan & Martinko 2009). Da die Hüllen und der Cortex der Sporen eine effektive Barriere gegen das Eindringen von Chemikalien bilden, sind Sporen weniger empfindlich gegenüber Bioziden als vegetative Zellen und entziehen sich somit möglicherweise auch einer Bekämpfungsmaßnahme (Russell 2003).

Weitere strukturelle Zelleigenschaften beeinflussen die Besiedlung terrestrischer Habitate durch Algen. MAAs und Scytonemin werden von aeroterrestrischen Algen und Cyanobakterien gebildet, um schädigende UV-Strahlung zu absorbieren (Büdel et al. 1997, Karsten et al. 2007b). Weiterhin scheinen fehlende oder kleine Vakuolen das Überleben trockener Perioden zu fördern (Ahmadijan 1967, Jacob et al. 1992). Wassermangel führt in Zellen zu osmotischem Stress. Als Schutz dagegen synthetisieren die Algen organische Osmolyte, wie Ribitol und Sorbitol (Gustavs et al. 2010). Diese Substanzen haben daneben weitere Schutzfunktionen, indem sie z.B. antioxidativ wirken (Yancey 2005). Auch Laccase-ähnlichen Enzymen, welche in Bodenalgen nachgewiesen wurden, werden entgiftende Eigenschaften zugesprochen (Otto et al. 2010). Die Enzymausstattung der Zellen beeinflusste die Wirksamkeit von Bioziden auf aquatische Algen (Tang et al. 1998). Es ist vorstellbar, dass zumindest einige der genannten Schutzmechanismen auch die Toleranz aeroterrestrischer Algen gegen Antialgenstrategien erhöhen, z.B. indem sie Radikale entgiften.

Die funktionellen Anpassungen an die Bedingungen im Habitat beziehen alle physiologischen Leistungen der Algen ein. An erster Stelle steht die außerordentlich schnelle Erholung der Photosynthese nach Austrocknung und Wiederbefeuchtung. Innerhalb weniger Minuten nach Befeuchtung trockener Zellen stieg deren maximale Quantenausbeute F_v/F_m an (Häubner et al. 2006). Begründet werden kann dies mit

der stabile Struktur der Chloroplasten aeroterrestrischer Grünalgen, welche sogar bei Austrocknung erhalten bleibt (Holzinger 2009). Auch an relativ trockenen Tagen waren zumindest geringe Quantenausbeuten an mit Grünalgen bewachsenen Fassaden messbar (Häubner et al. 2006). Echter photosynthetischer Gaswechsel wurde für *Apatococcus lobatus* bei bis zu 76 % Luftfeuchtigkeit nachgewiesen (Bertsch 1966). Flechten mit *Trebouxia* als Photobionten konnten sogar bei bis zu 70 % Luftfeuchtigkeit Kohlendioxid aufnehmen (Palmer & Friedmann 1990).

Die Fähigkeit, Wasserdampf zu absorbieren, ist eine wichtige Eigenschaft für das Wachstum außerhalb des aquatischen Milieus. Somit sind die Algen nicht auf Wasser bei Regen und Tau angewiesen und verlängern den Zeitraum ihrer Aktivität (Nienow 1996). Zahlreiche Studien zeigten, dass aeroterrestrische Algen Luftfeuchtigkeit nutzten, um ihren Wasserbedarf zu decken (Nienow 1996 und Referenzen darin, Gustavs et al. 2010, Gladis & Schumann 2011b). Über den physiologischen Mechanismus dieser Fähigkeit wird jedoch noch spekuliert. So könnten hydrophobe Anteile der Zellwand oder Osmolyte die Aufnahme von Wasserdampf ermöglichen (Nienow 1996 und Referenzen darin). Dennoch wuchsen Algen unter hoher Luftfeuchtigkeit deutlich langsamer als in flüssigem Medium (Gustavs et al. 2010, Abb. 4.2). In Wirksamkeitsnachweisen außerhalb von Suspensionen darf verlangsamtes Wachstum daher nicht als eine hemmende Wirkung der Antialgenstrategie fehlinterpretiert werden.

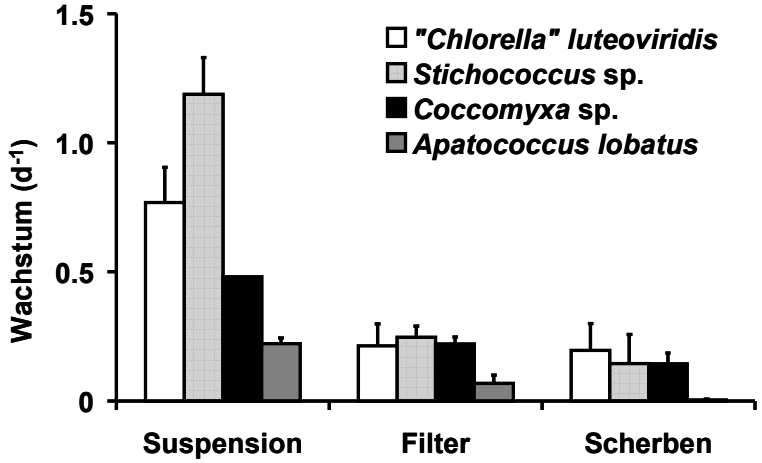

Abb. 4.2: Vergleich des Wachstums von aeroterrestrischen Grünalgenisolaten in Suspension und bei 100 % Luftfeuchte und täglicher Beregnung auf Glasfaserfiltern sowie auf Glasscherben. Dargestellt sind Mittelwerte von Positivkontrollen +/- Standardabweichungen (Suspension: n=3, Filter und Scherben: n=9).

Das Wachstum von aeroterrestrischen Grünalgen wird als euryök bezeichnet, da es durch eine hohe Toleranz gegenüber Schwankungen von Umweltfaktoren, wie Strahlungsintensität, Temperatur und Salzgehalt gekennzeichnet ist (Gustavs 2010). Algenbiofilme sind vor allem an schattigen Standorten zu finden, da hier der Wasserverlust relativ gering ist. Daher ist es auch nicht verwunderlich, dass die Algen schwachlichtangepasst sind (Lawrenz 2005). Bei geringen Lichtintensitäten ist der Chl *a*-Gehalt der Zellen hoch, um möglichst viel Strahlung zu absorbieren (Häder 1999). Vor hoher photosynthetisch aktiver Strahlung schützen sich Algen, nicht nur aeroterrestrische, durch photoprotektive Carotine und Xanthophylle. Über diese Pigmente geben die Algen überschüssige Energie in Form von Wärme ab. Auf Starklichtereignisse können aeroterrestrische Algen sehr schnell reagieren. Innerhalb von Minuten können sie über nichtphotochemisches Quenching Energie ableiten und verfügen über einen Xanthophyllzyklus, welcher schnell Lichtschutzpigmente synthetisiert (Demmig-Adams & Adams 1992, Lawrenz 2005). Dennoch sind einige aquatische Phototrophe noch besser an Strahlungsstress angepasst als aeroterrestrische Algen. Organismen des Supra- und Eulitorals sind durch Wellenfokussierungseffekte kurzfristig extrem hohen Photonenflussdichten ausgesetzt. Viele dort lebende Algen und Cyanobakterien schützen sich vor hoher Strahlung durch die Verschiebung der lichtsammelnden Antennen auf das Photosystem I, sogenannte State Transitions, und Phosphorylierung der Lichtsammelkomplexe (Falkowski & Raven 1997). Im Gegensatz dazu kann es beim Wachstum in tieferen Schichten des Biofilms zu Stress durch Lichtmangel kommen. Einige aeroterrestrische Algen scheinen ihren Stoffwechsel an fehlende Strahlung angepasst zu haben. *Apatococcus lobatus* überlebte bis zu 4 Monate in Dunkelheit vermutlich durch Mixotrophie (Gustavs et al. in prep.). Mixotrophe Organismen können sowohl phototroph als auch heterotroph wachsen und sind bei Lichtmangel gegenüber ausschließlich phototrophen Organismen im Vorteil.

Aeroterrestrische Algen wuchsen selbst bei winterlichen Temperaturen um den Gefrierpunkt noch vergleichsweise gut (Häubner et al. 2006). Diese Fähigkeit ermöglicht den Algen besonders in den Morgenstunden, in denen die höchste Feuchtigkeit auf den Oberflächen vorhanden ist, Stoffwechselaktivitäten, wie Photosynthese und Wachstum (Häubner 2004). Niedrige Temperaturen führen in Algen aber schnell zu Starklichtstress, da die photosynthetische Kohlenstoffassimilation durch Enzyme gesteuert wird. Es handelt sich damit um einen

temperaturabhängigen Prozess, während energieliefernde photochemische Prozesse temperaturunabhängig sind (Falkowski & Raven 1997). Geringe Temperaturen tolerieren Algen daher durch Anpassungen an hohe Lichtintensitäten, wie reduzierte Chl *a*- und erhöhte Carotenoidgehalte (Maxwell et al. 1994, vgl. oben). Temperaturen über 30°C werden von den Algen zwar toleriert, das Wachstum ist jedoch gehemmt (Häubner et al. 2006).

Aeroterrestrische Algen können sowohl unter limnischen als auch marinen Bedingungen wachsen. Hohe Salzgehalte führen dabei zu osmotischem Stress, welcher auch bei Austrocknung auftritt. Die Toleranz von hyperosmotischem Stress ist somit auch eine Anpassung an Wassermangel und wird durch die Synthese und Akkumulation von organischen Osmolyten realisiert (Gustavs et al. 2010). Während viele aeroterrestrische Algen Polyole speicherten, konnten diese Osmolyte in verwandten aquatischen Algen nicht nachgewiesen werden (Gustavs et al. 2011).

Verschiedenste Formen von Stress führen in lebenden Zellen zu oxidativem Stress, welcher als Ungleichgewicht zwischen Erzeugung und Entgiftung von ROS definiert ist. Algen sind oxidativem Stress jedoch auch schon physiologisch ausgesetzt. So werden schädliche ROS beim photosynthetischen Elektronentransport freigesetzt (Asada 1999). Aus diesem Grund haben Algen Anpassungen entwickelt, um oxidativen Stress zu tolerieren und Radikale zu entgiften. Dazu gehören etwa Antioxidantien, wie Glutathion, Ascorbat und Carotenoide, welche Sauerstoffradikale reduzieren. Auch Enzyme, wie Superoxiddismutase, Katalase und Peroxidasen, entgiften ROS. Zudem stehen reparierende Enzymsysteme zur Verfügung (Mallick & Mohn 2000). Viele Biozide verursachen oxidativen Stress, entweder indem sie direkt in die Bildung von ROS involviert sind oder indirekt durch die Hemmung des Stoffwechsels (Arora et al. 2002). Die Anpassungen an oxidativen Stress erhöhen die Toleranz von Algen gegen diese Antialgenstrategien.

Aufgrund der extremen Bedingungen in ihrem Habitat sind aeroterrestrische Algen Dauerstress ausgesetzt. Gestresste Zellen aktivieren verschiedene Schutzmechanismen, die sie gleichzeitig resistenter gegenüber Bioziden machen (Russel 2003). So produzieren viele Algen unter Stressbedingungen das Hormon Abscisinsäure, welches die Zellen auf vielfältige Weise schützt und vermutlich auch bedeutsam für die Austrocknungsresistenz aeroterrestrischer Algen ist. Es verändert die Permeabilität der Zellmembranen, erhöht die Aktivität von Katalasen und Peroxidasen und schützt die Zellen so vor oxidativen Schäden (Hartung 2010 und Referenzen darin).

Vermutlich kann dieses universell wirkende Hormon die Zellen auch vor der Wirkung einiger Antialgenstrategien schützen.

Als Folge von Stress sind Bakterien in der Lage in einen sogenannten „*Viable-but-non-culturable*"-Zustand wechseln (Potts 2001). In diesem Stadium teilen sich die Zellen zwar nicht mehr, sie können jedoch wieder in den kultivierbaren Zustand zurückgelangen. Für Bakterien ist dieses Stadium des Überlebens bereits gut untersucht (Oliver 2005). Ob ein solcher Zustand auch in Algen vorkommt, ist zwar noch nicht bekannt. Algen bilden jedoch funktionell ähnliche Dauerstadien, z.B. Akineten (Agrawal 2009, vgl. oben). In Wirksamkeitsnachweisen ist zu beachten, dass auch in Algen physiologische Inaktivität nicht mit Letalität gleichzusetzen ist.

Viele Biozide werden nur mit den aquatischen Modellorganismen aus den gesetzlich vorgeschriebenen Prüfverfahren, wie z.B. *Scenedesmus subspicatus*, getestet. Photokatalytische Materialien werden damit beworben, Algenbewuchs zu verhindern, obwohl ihre Wirksamkeit wissenschaftlich noch nicht belegt wurde. Vermutlich waren die nachgewiesene Aktivität gegenüber Bakterien und chemischen Verbindungen die Motivation für diese Versprechen (z.B. Mills & LeHunte 1997). Aufgrund ihrer Anpassungen an die extremen Bedingungen in ihrem Habitat ist zu erwarten, dass aeroterrestrische Algen sehr resistent gegenüber Bekämpfungsmaßnahmen sind. Folglich kann die Wirksamkeit einer Antialgenstrategie gegenüber Bakterien oder aquatischen Algen keinesfalls auf aeroterrestrische Algen extrapoliert werden.

4.2 Physiologie aeroterrestrischer Algenbiofilme

Insbesondere das Leben im Biofilm prägt die Eigenschaften von aeroterrestrischen Algen. Biofilme sind miteinander kooperierende Gemeinschaften von Mikroorganismen, die durch eine Matrix aus EPS aneinander und ans Substrat gebunden sind. Aeroterrestrische Biofilme können aus Bakterien, Algen, Cyanobakterien, Pilzen und Flechten zusammengesetzt sein (Gorbushina 2007, Abb. 4.3). Im Gegensatz zu den relativ homogenen aquatischen Biofilmen sind aeroterrestrische Biofilme durch zusammenklumpende Mikrokolonien vor allem in geschützten Bereichen des Substrates, wie Mulden, Spalten und Poren, gekennzeichnet (Gorbushina 2007). In aquatischen Biofilmen können die EPS bis zu 90 % des gesamten organischen Kohlenstoffes ausmachen (Donlan 2002). Aeroterrestrische Algenbiofilme scheinen jedoch deutlich kompakter und durch einen höheren Organismenanteil gekenn-

zeichnet zu sein (Gustavs 2010). Die EPS bestehen hauptsächlich aus Polysacchariden und binden große Wassermengen (Donlan 2002). Daneben enthalten sie Proteine, Glycoproteine und Proteoglycane (Mostaert et al. 2009). In Folge von wechselnder Austrocknung und Wiederbefeuchtung unterliegt die EPS-Matrix einem ständigen Wechsel aus Kontraktion und Entspannung (Gorbushina 2007).

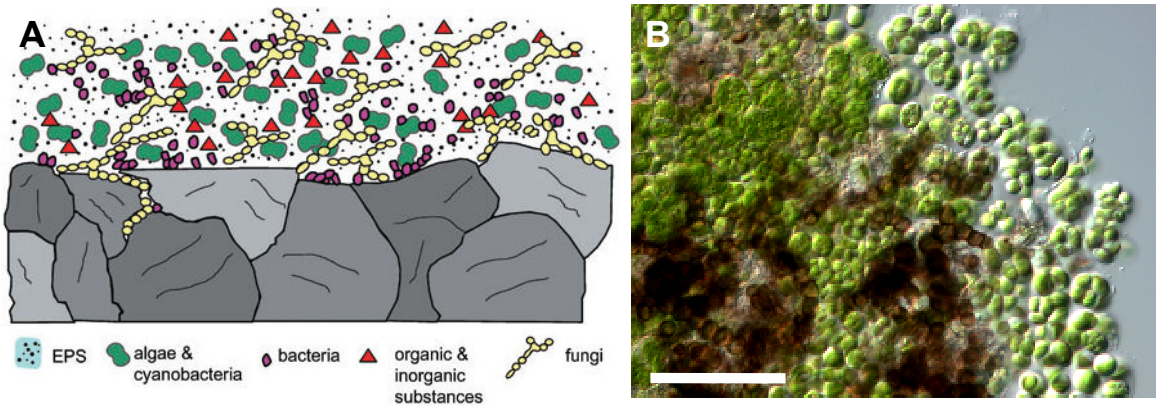

Abb. 4.3: A) Schematischer Aufbau eines aeroterrestrischen Biofilms, in dem die Mikroorganismen (Algen, Cyanobakterien, Pilze und Bakterien) in einer Matrix aus EPS eingeschlossen sind (Quelle: Gorbushina 2007). B) Mikrofotographische Aufnahme der Biofilmgemeinschaft aus Grünalgen, Pilzen und Bakterien. (Foto: M. Görs, Maßstab 50 µm).

Biofilme sind heterogene Strukturen, an und in denen chemische und physikalische Gradienten ausgebildet werden. Für phototrophe Biofilme ist vor allem der Strahlungsgradient von Bedeutung. Gustavs et al. (in prep.) detektierten in einem aeroterrestrischen Biofilm eine Abschwächung der Strahlung in den obersten 100 µm um 90 %. Je nach ihrer Position im Biofilm bedeutet dies für die Algen sehr unterschiedliche Bedingungen. Während äußere Zellen eher Starklichtstress ausgesetzt sind, können innen liegende Zellen ihren phototrophen Stoffwechsel kaum aufrechterhalten. In marinen Biofilmen wurde beobachtet, dass nur die oberen Schichten photosynthetisch aktiv waren und es darunter zu anaeroben Zonen kam (Leadbeater & Callow 1992). Aufgrund der hohen Kompaktheit des Biofilms und fehlender lichtstreuender Partikel betrug die photische Zone in einem aeroterrestrischen Biofilm weniger als 0,2 mm, während sie in marinen Biofilmen bis zu 3 mm betragen kann (Garcia-Pichel & Bebout 1996, Gustavs et al. in prep.). Da aeroterrestrische Algen sich nicht aktiv in obere Schichten bewegen können, vermu-

teten Gustavs et al. (in prep.), dass Algen in tiefen Biofilmzonen durch mixotrophe Ernährungsweise wachsen (vgl. Kap. 4.1).

In Biofilmen kommt es zu Mikrozonen, in denen die Konzentrationen von Sauerstoff, Kohlendioxid, Nährstoffen und gelöstem organischem Kohlenstoff räumlich und zeitlich variieren können (Leadbeater & Callow 1992). Die Produktivität der Algen kann dadurch immer wieder limitiert werden. Die Remineralisierung von Nährstoffen in aeroterrestrischen Biofilmen ist noch nicht untersucht worden. Vermutlich werden Substrate, ähnlich wie im aquatischen Milieu, über extrazelluläre Enzyme in der Biofilmmatrix hydrolysiert (Thompson & Sinsabaugh 2000). Der Kohlendioxidbedarf der Algen scheint größtenteils durch die Respiration heterotropher Bakterien gedeckt zu werden (Kühl et al. 1996).

Die Funktion von Biofilmen in terrestrischen Habitaten ist in erster Linie die Pufferung der äußeren Bedingungen. Innerhalb der EPS werden extreme Temperaturen, Strahlung und Austrocknung gedämpft (Mager & Thomas 2011). Ferner fungieren die EPS als Wasserspeicher, wirken osmotisch und erleichtern den Zugriff auf Wasserdampf in der Atmosphäre (Gorbushina 2007). An den EPS absorbieren Nährstoffe, welche mit Staub und Regenwasser transportiert werden (Callow & Callow 2006). Das Wachstum im Biofilm scheint innen liegende Zellen vor Schaden von außen zu schützen und so ein Überleben und Wiederaufleben einer Kolonie bei verbesserten äußeren Bedingungen zu ermöglichen (Gustavs 2010). Auch für die Verbreitung der aeroterrestrischen Algen ist das Vorkommen in einer Biofilmmatrix vermutlich von Bedeutung. Aeroterrestrische Organismen verbreiten sich wahrscheinlich nicht als Einzelzellen, sondern als zusammenhängende Aggregate, welche über die Luft transportiert werden, sich an ein Substrat heften und unter günstigen Bedingungen anwachsen (Gorbushina 2007).

Der erste Schritt in der Bildung von Biofilmen ist die Anheftung an einem Substrat. Die Adhäsion der Mikroalgen wird durch die Oberflächeneigenschaften des Substrats, aber auch die physiko-chemischen Charakteristika der Algen, vermittelt. Adhäsion ist dabei der Schlüsselschritt im Oberflächenbiofouling. Da die bakterielle Adhäsion oft mit gesundheitlichen Gefahren verbunden ist, wurde sie bereits gut untersucht (Überblick z.B. in Donlan 2002, Kokare et al. 2009). Arbeiten zur Algenadhäsion beschränken sich zumeist auf aquatische Biofilme (z.B. Holland et al. 2004). Für aeroterrestrische Algen ist jedoch bekannt, dass amyloide Fibrillen im ausgeschiedenen „Klebstoff“ den Algen eine feste Bindung an unterschiedlichsten Oberflächen

ermöglichen (Mostaert et al. 2009). Barberousse et al. (2007) bestimmten zudem hydrophobe Interaktionen als adhäsionsfördernd. Die Adhäsion der Algen ist sehr stark und mechanisch kaum zu lösen (Mostaert et al. 2009, eigene Erfahrungen).

Die Organismen in Biofilmen interagieren intensiv miteinander und sichern so ihr Überleben und ihren Erfolg. Bei der Bildung eines Biofilms werden durch die Anheftung der ersten Zellen die Bedingungen für das Festsetzen weiterer Mikroorganismen und Nährstoffe erleichtert und das Wachstum gefördert. Gorbushina (2007) beschrieb die Biofilmbildung daher als autokatalytisch. In aeroterrestrischen Biofilmen wurden synergistische Beziehungen zwischen den Organismengruppen beobachtet. So kooperierten phototrophe Algen und heterotrophe Pilze mit ähnlichen Funktionen wie in der Flechtensymbiose (Gorbushina et al. 2005). Die Pilze wurden mit organischen Kohlenstoffverbindungen versorgt, während die Algen vermutlich durch die melanisierten Pilze geschützt wurden.

Es ist wahrscheinlich, dass die Organismen in Biofilmen auch miteinander kommunizieren. Die Kommunikation zwischen einzelnen Bakterienzellen über Signalmoleküle wird als *Quorum Sensing* bezeichnet. Dieses spielt beispielsweise bei der Adhäsion und Ablösung von bakteriellen Biofilmen eine Rolle (Donlan 2002). Während eine solche Kommunikation zwischen Algen noch nicht beobachtet wurde, können Cyanobakterien über *Quorum Sensing* miteinander in Verbindung stehen (Vassilakaki & Pflugmacher 2008). Die Grünalge *Chlamydomonas reinhardtii* konnte die bakterielle Kommunikation imitieren und dadurch stören (Teplitski et al. 2004). Umgekehrt wurde auch die Adhäsion von Zoosporen der Makoalge *Ulva* durch bakterielle Signalmoleküle beeinflusst (Callow & Callow 2006). Die Bedeutung von *Quorum Sensing* in der Anheftung und Entwicklung von aeroterrestrischen Biofilmen wurde bisher noch nicht erforscht.

Die Art der Kultivierung aeroterrestrischer Algen im Labor, im Biofilm oder in Suspension, beeinflusst die Eigenschaften der Mikroorganismen. Die biofilmassoziierten Zellen unterscheiden sich von Zellen, die in Suspensionen wachsen, neben der Bildung einer Matrix aus EPS vor allem durch verringerte Wachstumsraten (Donlan 2002, Abb. 4.2). Für Bakterien wurde das An- und Abschalten mehrerer Gene beobachtet, sobald Bakterien in einem Biofilm wuchsen (Callow & Callow 2006). Auch die Zusammensetzung der bakteriellen EPS unterschied sich in Suspension von der im Biofilm (Allison et al. 1998). Für Wirksamkeitsnachweise ist die Untersuchung von Biofilmen besonders bedeutend. Die EPS erhöhen die Resistenz der Organismen,

weil sie als Diffusionsbarriere den Transport der schädigenden Moleküle durch den Biofilm verhindern (Donlan 2002). Daneben kann Stress, wie z.B. photooxidativer Stress, die Empfindlichkeit gegenüber Bioziden erhöhen (Foyer et al. 1994, Guasch & Sabater 1998). Die heterogenen Biofilme verändern die simplen Dosis-Wirkungsbeziehungen von suspendierten Zellen zu zeitlich und räumlich wechselnden Beziehungen (Hall-Stoodley et al. 2004). Eine Bewertung der Wirksamkeit von Antialgenstrategien ist folglich nur möglich, wenn die Wirkung auf den geschützten Biofilm betrachtet wird.

4.3 Nachweis der Wirksamkeit von Antialgenstrategien

Die Mechanismen in Biofilmen können nicht verstanden werden, wenn die Physiologie der Organismen nur in Suspension untersucht wird (Callow & Callow 2006). Bei der Bewertung von Antialgenstrategien muss daher zuerst die Frage nach der Physiologie der betreffenden Organismen beantwortet werden. Und nur wenn die Wirkung von Antialgenstrategien auf natürliche und Modellbiofilme untersucht wird, kann auch eine Aussage zu ihrer Wirksamkeit an Baumaterialien getroffen werden. Während bakterielle Biofilme in den letzten Jahren vermehrt im Fokus der Forschung standen (z.B. Kokare et al. 2009), ist die Erforschung von Algenbiofilmen noch am Anfang. So gibt es für phototrophe Biofilme bisher keine Standardmethoden, die ihre Quantifizierung und Charakterisierung ermöglichen. Im Folgenden werden die Schwierigkeiten der Untersuchung phototropher Biofilme dargelegt und Lösungen für Wirksamkeitsnachweise diskutiert.

In der Freibewitterung wird das Algenwachstum auf Materialproben über mehrere Monate bis Jahre beobachtet. Die Untersuchung natürlicher Biofilme im Freiland bietet den Vorteil, dass die Materialien einem „echten“ Infektionsdruck ausgeliefert sind. Die Biofilmbildung erfolgt unter realistischen Bedingungen und in Echtzeit durch die Zielorganismen der Antialgenstrategien, die aeroterrestrischen Mikroalgen. Diese Untersuchungen sind jedoch sehr langwierig. Zudem wird das Wachstum der Algenbiofilme sehr stark durch das zeitlich und örtlich variable Klima bestimmt (Gladis et al. in prep.). Strahlungsdosis, Niederschlag, Temperatur und Windgeschwindigkeit sowie -richtung beeinflussen die Verdunstung an Oberflächen. Auch Luftschadstoffe, Vorkommen von abweidenden Tieren (Schnecken, Läuse), benachbarte Bebauung oder Bewuchs, Gebäudeklima und bauphysikalische wie chemische

Eigenschaften des Materials bestimmen das Algenwachstum im Freiland (Seaward 1979). Diese Vielzahl von Einflussfaktoren führt dazu, dass Freibewitterungen kaum reproduzierbar und wenig auf die vielfältigen Einsatzbedingungen übertragbar sind.

Durch die Simulation der natürlichen Biofilmbildung im Labor können aeroterrestrische Algenbiofilme unter kontrollierten Bedingungen untersucht werden. Dabei lässt sich die Wirksamkeit einer Antialgenstrategie nur durch den Vergleich mit ungestörten Biofilmen, d.h. mit Kontrollen ohne Wirkstoff oder aktive Oberfläche, ableiten. In Laborversuchen muss daher zuerst das Wachstum von Positivkontrollen, also Algenbiofilmen auf inaktiven Materialien, gewährleistet werden. Das Wachstum der Algen im Biofilm ist heterogen und die Wachstumsraten können stark schwanken (Gladis & Schumann 2011b). Des Weiteren kann das Wachstum einiger aeroterrestrischer Algen, wie *Apaptococcus lobatus*, so langsam sein, dass wachstumshemmende Wirkungen nicht erkannt werden können (Abb. 4.2). Um fehlendes Wachstum auf eine Antialgenstrategie zurückzuführen, ist es daher erforderlich, die Schwankungen des ungestörten Biofilmwachstums zu kennen. Für die Etablierung eines Wirksamkeitsnachweises als Standardmethode, sollte das Wachstum der Algenbiofilme unter konstanten Laborbedingungen schnell und reproduzierbar erfolgen. Ein Kompromiss aus naturnahen und das Wachstum fördernden Bedingungen ermöglicht das Wachstum von Biofilmen innerhalb von Tagen bis Wochen. Naturnahe Bedingungen sollen die Bildung von echten Biofilmen gewährleisten. Da die EPS-Synthese durch Wassermangel induziert wird (Roberson & Firestone 1992), wurde angenommen, dass Biofilme gebildet werden, wenn die Algen ihren Wasserbedarf, wie unter natürlichen Bedingungen, aus Wasserdampf decken müssen. Das Wachstum in Biofilmen stellt eine Anpassung an die aeroterrestrische Lebensweise dar, die einen höheren Energieverbrauch der Zellen zur Folge hat, und zu Lasten hoher Wachstumsraten geht (Friedmann & Ocampo-Friedmann 1984, Donlan 2002). Daher sollte die Wasserversorgung der Algen möglichst hoch sein. Geeignete Wachstumsraten zwischen 0,14 und 0,20 d^{-1} wurden gemessen, wenn die Algen bei 100 % Luftfeuchte inkubierten und täglich künstlich „beregnet“ wurden (Abb. 4.2). Lediglich eines von vier Algenisolaten konnte innerhalb von zwei Wochen keine sichtbaren Biofilme bilden. Auf Glasscherben ließen sich die Algenzellen nicht von der Oberfläche abspülen und waren folglich durch EPS fest ans Material gebunden (Gladis & Schumann 2011b).

Die Strahlungsbedingungen sind zum einen für die Photosynthese der Algen und im Fall der Photokatalyse zum anderen für deren Aktivierung von Bedeutung.

Strahlungsstress scheint die Toleranz gegenüber Antialgenstrategien zu senken, da er wie auch andere ungünstige Bedingungen und zahlreiche Biozide zu photooxidativen Schäden in den Zellen führt (Foyer et al. 1994). So erhöhte Starklichtstress die Empfindlichkeit von Rotalgen gegenüber Bioziden (z.B. Reis et al. 2011). Vermutlich sind die Verteidigungsstrategien der Algen bei zusätzlichem Stress durch Biozide schneller ausgelastet. Gleichzeitig erhöhten Schwachlichtbedingungen die toxische Wirkung des Herbizides Atrazin, da dieses die Energieübertragung hemmte (Mayer et al. 1998). In Toxizitätsuntersuchungen haben optimale Wachstumsbedingungen sowohl hohe Wachstumsraten der Kontrollen als auch reproduzierbare Ergebnisse zur Folge (Mayer et al. 1998). Um die Wirksamkeit einer Antialgenstrategie auf den Biofilm beurteilen zu können, müssen die Bedingungen für alle Algen gleich sein. Gegenseitige Beschattung kann das Wachstum einiger Algen inhibieren und ihre Empfindlichkeit verändern. Sollen photokatalytisch aktive Materialien untersucht werden, muss zusätzlich berücksichtigt werden, dass dichte Biofilme gleichzeitig die photokatalytisch aktive Oberfläche bedecken und ihre Aktivierung durch Photonen verhindern. Daher müssen die Algenbiofilme für eine Wirksamkeitsprüfung sehr dünn sein.

Die meisten Photokatalysatoren werden durch UV-Strahlung aktiviert (Mills & LeHunte 1997). Hohe UV-Intensitäten erhöhten die Effizienz bei der Abtötung von Bakterien (Benabbou et al. 2007). Um die Ergebnisse von Wirksamkeitsnachweise auf die Praxis übertragen zu können, sollte die Intensität der Strahlung die Bedingungen im beschatteten Habitat der Algen widerspiegeln und entsprechend gering sein. Dennoch können auch schon geringe UV-Dosen Organismen schädigen. In zahlreichen Wirksamkeitsnachweisen mit Bakterien blieb die UV-Empfindlichkeit der Organismen unbeachtet (z.B. Kiwi & Nadtochenko 2005, Benabbou et al. 2007). Für den Nachweis der Wirkung photokatalytisch aktiver Materialien darf die Wirkung der UV-Strahlung jedoch nicht mit einer photokatalytischen Wirkung verwechselt werden. Mit Hilfe von Kontrollen lassen sich die einzelnen Wirkungen quantifizieren. Das Auftreten synergistischer Wirkungen von Photokatalyse und UV-Strahlung ließe sich durch weitere Kontrollen detektieren. Dafür müssten zusätzlich durch sichtbares Licht aktivierte photokatalytische Materialien untersucht werden (z.B. KRONOS vlp 7000).

Art- bzw. isolatspezifische strukturelle und funktionelle Eigenschaften von Algen beeinflussen ihre Empfindlichkeit in Wirksamkeitsnachweisen und damit die

Ergebnisse dieser Untersuchungen (Bengtson-Nash et al. 2005). Aeroterrestrische Algen sind aufgrund ihrer zahlreichen physiologischen Anpassungen wahrscheinlich besonders tolerant (vgl. Kap. 4.1 und 4.2). Um die Übertragbarkeit der Ergebnisse von Wirksamkeitsnachweisen auf die Umwelt bzw. Anwendung zu gewährleisten, sollten die verwendeten Modellorganismen für das Ökosystem repräsentative Vertreter sein (Hörnström 1990). Die in dieser Arbeit untersuchten Isolate gehören zu den abundantesten aeroterrestrischen Mikroalgen (Gustavs 2010). Im Gegensatz zu ökotoxikologischen Untersuchungen, die die Gefahren eines Stoffes in der Umwelt bestimmen, werden für den Nachweis der Wirksamkeit vielmehr tolerante statt besonders empfindliche Testorganismen benötigt. Da die Empfindlichkeit artspezifisch variieren kann, sollte eine Auswahl von mehreren Arten untersucht werden, um realistische Ergebnisse zu erhalten (Blanck et al. 1984, Rojicková-Padrtová & Marsálek 1999).

Die Wirksamkeit einer Antialgenstrategie kann über die Hemmung des Algenwachstums sehr sensitiv detektiert werden (Gladis et al. 2010). Die Grundlage für die Wachstumshemmung bildet die Quantifizierung der phototrophen Biomasse. Während die Biomasse suspendierter Mikroorganismen sehr leicht, z.B. über mikroskopische Zellzählungen, bestimmt werden kann, sind Biofilme deutlich schwieriger zu quantifizieren. Bei der klassischen Methode der Biomassequantifizierung bakterieller Biofilme wird die Zahl der koloniebildenden Einheiten auf Agarplatten bestimmt, nachdem die Organismen von der Oberfläche abgelöst wurden. Da jedoch weniger als 1 % der Bakterien als kultivierbar gelten, selektiert diese Methode bestimmte Arten aus natürlichen Biofilmen (Amann et al. 1995). Die in Untersuchungen zur photokatalytischen Wirksamkeit gegen Bakterien üblichen Abklatschverfahren, in denen Nährböden an die Testmaterialien gedrückt werden (ISO 27447), erfordern zusätzlich die Ablösung der Organismen vom Testmaterial. Zudem sind die Zellzahlen der Kultivierung auch schnellwachsender Bakterienisolate deutlich geringer als die mit mikroskopischen Verfahren ermittelten Biomassen, denn nicht alle Zellen wachsen zu einer sichtbaren Kolonie (Nakajima et al. 2005). Die Theorie der „*Great Plate Count Anomaly*“ beschreibt, dass Laborbedingungen die natürlichen Bedingungen nicht exakt wiedergeben können und die Biomassen mit kultivierungsabhängigen Methoden daher unterbestimmt werden (Staley & Konopka 1985). Da festhaftende Organismen und solche, die keine Kolonien bilden, nicht erfasst werden, sind diese Verfahren stark fehlerbehaftet. Außerdem werden verschiedene mikrosko-

pische Techniken von der klassischen Lichtmikroskopie über Epifluoreszenzmikroskopie bis hin zur Elektronenmikroskopie für die Quantifizierung von bakteriellen Biofilmen verwendet. Dabei können Zellen, Zellbestandteile oder die umgebende EPS mit Farbstoffen visualisiert werden (zusammengefasst in Hannig et al. 2010). Als Biomasseparameter dienen Proteine, Kohlenstoff, EPS oder Trockenmasse (z.B. Schumann & Rentsch 1998, Staudt et al. 2004).

Die Pigmentierung phototropher Biofilme bietet gegenüber bakteriellen Biofilmen Vorteile bei der Quantifizierung. So sind grüne, rote oder dunkle Verfärbungen schon makroskopisch erkennbar und erlauben eine schnelle semiquantitative Erfassung des Bewuchses (Donner et al. 2002, Schumann et al. 2004, Tab. 4.1). Jedoch ist dieses Verfahren subjektiv und durch Helligkeit, Reflektion, Materialfarbe und -feuchtigkeit sowie andere Verfärbungen auf der Oberfläche beeinflusst. Mit Hilfe der softwarebasierten Bildanalyse von Fotografien bewachsener Flächen können die grünen Verfärbungen der Algenbiofilme objektiv quantifiziert werden, jedoch stören die oben genannte Faktoren weiterhin eine exakte Bestimmung (Gladis & Schumann 2011a).

Eine quantitative Biomassebestimmung erfolgt über den Chl *a*-Gehalt der Algen. Obwohl dieser artspezifisch und durch Umweltfaktoren beeinflusst ist, kann aus der Chl *a*-Konzentration auf die Algenbiomasse geschlossen werden (Schumann et al. 2005). Die Bestimmungsgrenze dieses Verfahrens liegt in Abhängigkeit von der beprobten Fläche sehr niedrig (beispielhaft für das Isolat *Stichococcus* sp. berechnet bei etwa 2 x 10^6 Zellen cm^{-2} für eine Beprobungsfläche von 1 cm^2) und ist im oberen Messbereich nicht begrenzt, da die Extrakte verdünnt werden können (Tab. 4.1). Um Chl *a* zu extrahieren, werden die Algen und oft auch Teile des Materials vollständig von der Oberfläche entfernt. Eine weitere Entwicklung des Biofilmwachstums kann mit dieser invasiven Methode somit nicht beobachtet werden.

Optische Methoden, die auf der Chl *a*-Fluoreszenz basieren, sind sehr gut geeignet, um die Biomasse dünner und junger Biofilme nicht-invasiv zu bestimmen, stoßen jedoch bei mehrschichtigen Biofilmen, in denen sich die Zellen gegenseitig beschatten, an ihre Grenzen (Barranguet et al. 2004). Mittels PAM-Fluorometrie wird die Chl *a*-Fluoreszenz, welche proportional zu seiner Konzentration und damit der Biomasse ist, gemessen (Eggert et al. 2006). Diese Methode ist genauer als die semiquantitative Bildanalyse und hat keinen störenden Einfluss auf den Biofilm, so dass sie sehr gut geeignet ist, um die Entwicklung von Algenbiofilmen im Freiland zu

Tab. 4.1: Merkmale von Verfahren zur Biomassequantifizierung phototropher Biofilme. Die Entsprechung der Messgrenzen sind für das Grünalgenisolat *Stichococcus* sp. (ROS 55/3) berechnet worden. Umrechnung aus zellspezifischem Chl *a*-Gehalt (235 fg Zelle^{-1}, Eggert et al. 2006) bzw. zellspezifischer Chloroplastengröße (2,7 µm^2 Zelle^{-1}, eigene Berechnungen).

	Visuelle Bewertung nach Schumann et al. 2004	**Chl *a* – Extraktion nach Schumann et al. 2005**	**PAM-Fluorometrie nach Eggert et al. 2006**	**Mikroskopische Bildanalyse nach Gladis & Schumann 2011b**
Bestimmungsgrenze	Gering intensiv und vereinzelte Grünfärbung **entspricht 2 x 10^6 Zellen cm^{-2}**	5 mg Chl *a* m^{-2} **entspricht 2.1 x 10^6 Zellen cm^{-2}**	0,1 V **entspricht 1.5 x 10^6 Zellen cm^{-2}**	1 Zelle je Foto **entspricht 2 x 10^3 Zellen cm^{-2}**
Oberer Messbereich	Deutliche und größere Veralgung **entspricht 17 - 133 x 10^6 Zellen cm^{-2}**	**∞** **∞**	1.5 V **entspricht 20 x 10^6 Zellen cm^{-2}**	Einschichtiger Biofilm **entspricht 20 x 10^6 Zellen cm^{-2}**
Größe einer begutachteten Fläche	Ca. 10 cm^2	1 cm^2	28 mm^2	0,05 mm^2
Zahl der begutachteten Flächen	1	1	5	30
Vorteile	Einfach	Objektiv	Nicht-invasiv und objektiv	Niedrige Bestimmungsgrenze, nicht-invasiv und objektiv
Nachteile	Subjektiv	Invasiv	Enger Messbereich	Aufwendig

untersuchen (Gladis & Schumann 2011a). In Laboruntersuchungen konnten auf diese Weise Wachstumsraten von Biofilmen berechnet werden (Gladis et al. 2010). Jedoch ist der Bereich, in dem der Zusammenhang zwischen Chl *a*-Fluoreszenz und Biomasse linear ist, relativ klein und entspricht nur etwa 1,5 - 20 x 10^6 Zellen cm^{-2} *Stichococcus* sp. (Eggert et al. 2006, Tab. 4.1). In diesem Bereich ist die Biomasse hoch genug, um reproduzierbar quantifiziert zu werden und niedrig genug, um gegenseitige Beschattung der Algen auszuschließen. Für die Wachstumsbestimmung nach dieser Methode, müsste die Biomasse des Inokulums auf den Oberflächen im unteren Bereich liegen. Auf Oberflächen verteilen sich die Algen jedoch heterogen. Dadurch

kann die geringe Biomasse, die für die Bildung eines nur dünnen Biofilms erforderlich ist, an den meisten Stellen anfangs unter der fluorometrischen Bestimmungsgrenze liegen (Eggert et al. 2006, Tab. 4.1). Infolgedessen ist diese Methode zu wenig sensitiv für Wirksamkeitsnachweise an wachsenden Biofilmen. Im Gegensatz zur Bestimmungsgrenze liegt die Nachweisgrenze der PAM-Messung bei etwa 0,03 V, das bedeutet Algen werden qualitativ, jedoch nicht quantitativ bestimmt. Visuell sind die phototrophen Biofilme in diesem Bereich nicht eindeutig erkennbar. Kleinere Werte entstehen durch Reflexionen und Signalrauschen und können nicht sicher auf die Anwesenheit von Algen zurückgeführt werden (eigene Erfahrungen). In Freilanduntersuchungen ist diese Methode somit geeignet für den Nachweis erster Spuren von Algenbiofilmen, die visuell noch nicht erfasst werden können (Gladis & Schumann 2011a).

Um geringe Algenbiomassen auf Oberflächen zu quantifizieren, wird, wie schon für bakterielle Biofilme, auf mikroskopische Techniken mit niedrigen Bestimmungsgrenzen zurückgegriffen. Bei dem in dieser Arbeit entwickelten Verfahren wird das Chl *a* der Mikroalgen epifluoreszenzmikroskopisch detektiert, indem die Chl *a*-Fluoreszenz der Algen auf den Oberflächen mit blauer Strahlung (460 - 490 nm) angeregt wird (Gladis & Schumann 2011b). Durch die Aufnahme von Mikrofotografien und anschließender Bildanalyse werden der Anteil der bewachsenen Fläche und im zeitlichen Verlauf schließlich das Wachstum auf den Oberflächen berechnet. Selbst Biofilme, die so dünn sind, dass sie mit bloßem Auge nicht erkennbar sind, können auf diese Weise quantifiziert werden (Tab. 4.1). Gleichzeitig reicht der obere Messbereich theoretisch bis zu einem geschlossenen, jedoch noch einschichtigen Biofilm. Für das Beispiel *Stichococcus* sp. entspricht ein solcher Biofilm einer Zellzahl von 37 x 10^6 Zellen cm^{-2}. Da die Zellen sich jedoch nicht homogen in einer Ebene verteilen, muss die tatsächliche obere Bestimmungsgrenze vielmehr bei etwa 20 x 10^6 Zellen cm^{-2}, wie für die fluorometrische Chlorophyllmessung, liegen (eigene Erfahrungen, Tab. 4.1).

Biofilme sind räumlich heterogene Strukturen, deren Biomassen auf einer Oberfläche stark variieren können. Um die gesamte Biomasse auf einem Prüfkörper möglichst fehlerfrei zu quantifizieren, ist es daher erforderlich, dass viele Stellen der Oberfläche begutachtet werden. Vor allem bei kleinen begutachteten Flächen, muss die Biomasse also an mehr Stellen bestimmt werden. Für reproduzierbare Ergebnisse wurden im mikroskopischen Verfahren bis zu 30 kleine Bilder (je 0,05 mm^2) einer

16 cm^2 großen Oberfläche aufgenommen, während 5 größere Stellen (je 28 mm^2) eines 4,9 cm^2 großen Filters PAM-fluorometrisch quantifiziert wurden (Tab. 4.1). Für die Erfassung der Heterogenität von phototrophen Biofilmen stehen bildgebende PAM-Verfahren zur Verfügung. Deren Messbereiche lagen für die Alge *Stichococcus* sp. zwischen 0,9 und 20 x 10^6 Zellen cm^{-2} (Eggert et al. 2006).

Die geringen Biomassen, die erforderlich sind, um die Wirksamkeit von Antialgenstrategien über das Wachstum nachzuweisen, waren mit den etablierten Methoden nicht zu quantifizieren. Daher wurde in dieser Arbeit ein neue Methode entwickelt, mit der das Wachstum dünner Algenbiofilme sowohl objektiv als auch nicht-invasiv quantifiziert werden kann. Wird jedoch Chl *a* als Biomasseparameter verwendet, müssen folgende Punkte beachtet werden:

(1) Die hier angegebenen Zelldichten beziehen sich auf das Isolat *Stichococcus* sp. und müssen für andere Arten neu bestimmt werden. Eggert et al. (2006) berechnete ähnliche Kalibriermodelle für vier weitere Grünalgenisolate. Der gemessene Parameter F_0 spiegelt die Chlorophyllfluoreszenz der lichtsammelnden Antennen des Photosystem II wider (Krause & Weis 1991). Der Photosyntheseapparat von verschiedenen Grünalgen ist ähnlich aufgebaut und somit unterschieden sich die Modelle nicht wesentlich voneinander. Im Gegensatz dazu muss für andere Algengruppen mit anderem Aufbau des Photosyntheseapparates der Zusammenhang zwischen Chlorophyllfluoreszenz und Biomasse neu ermittelt werden. Die Chl *a*-Fluoreszenz der Algen wird durch Variationen der Antennengröße, ihrer Anordnung bei Zellgrößenänderung sowie intrazellulärer Pigmentkonzentrationen beeinflusst (Mitchell & Kiefer 1988, Krause & Weis 1991). Der Einfluss von Carotenoiden als Lichtschutzpigmente oder anderen membranassoziierten Substanzen mit hohen Absorptionskoeffizienten muss zudem noch überprüft werden. Daneben stören hohe Anteile von anderen Mikroorganismen wie Bakterien und Pilzen sowie verkrustete Anteile die Biomassequantifizierung von Biofilmen, indem sie die Chl *a*-Fluoreszenz abschwächen.

(2) Im Freiland kann die PAM-fluorometrisch gemessene Grundfluoreszenz F_0 aufgrund der tagsüber nicht durchführbaren Dunkelanpassung nicht gemessen werden. Jedoch korrelierte die Chlorophyllfluoreszenz unter aktinischem Licht F_t mit der in der Nacht gemessenen Fluoreszenz F_0 in natürlichen Biofilmen signifikant miteinander (R_S = 0,83; $p<0,001$). Somit ist die Biomassequantifizierung natürlicher Algenbiofilme über F_t mit geringem Aufwand durchführbar (Gladis & Schumann 2011a).

(3) Stress, verursacht z.B. durch Trockenheit oder Starklicht, verändert den physiologischen Zustand der Algen sowie auch die Chl *a*-Fluoreszenz (Franklin et al. 1992, Pearson et al. 2000). Auch die Antialgenstrategien, welche das Algenwachstum unterdrücken sollen, beeinflussen somit die Chl *a*-Fluoreszenz. Bei der Verwendung dieses Biomasseparameters sollte überprüft werden, ob die Antialgenstrategie zu Schwächung oder Anstieg des Fluoreszenzsignals in den Zellen führt.

(4) Eine Antialgenstrategie ist dauerhaft nur wirksam, wenn die Algen an der Oberfläche nicht mehr wachsen können. Um nachzuweisen, dass die Algen nicht nur physiologisch inaktiv sind, keine toleranten Einzelzellen überlebt haben und somit keine Erholung mehr möglich ist, müssen Vitalitätsparameter, welche den Zelltod detektieren, untersucht werden. Eine mögliche Methode ist der epifluoreszenzmikroskopische Nachweis permeabilisierter Zellen, z.B. mit dem Farbstoff SYTOX® Green, welcher auch auf Oberflächen angewendet werden kann (Gladis & Schumann 2011b, Abb. 4.4). Dieser Farbstoff differenziert zwischen geschädigten und vitalen Algenzellen, indem er nur durch permeable Zellmembranen dringt und somit tote Zellen markiert. Die Membranintegrität geht erst in einem späten Stadium des Zelltodes verloren. Die Zellen können sich somit von einem Verlust der Membranintegrität nicht mehr erholen (Veldhuis et al. 2001).

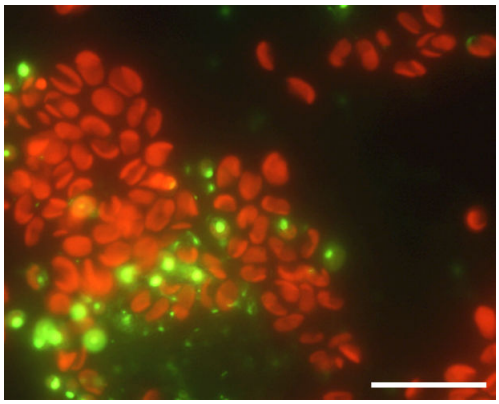

Abb. 4.4: Epifluoreszenzmikroskopische Diskriminierung zwischen vitalen Zellen mit roter Chl *a*-Fluoreszenz und toten Zellen, in denen der Farbstoff (SYTOX® Green) die permeablen Membranen passieren konnte und an die DNA bindet, in einem Biofilm von *Coccomyxa* sp. (SAG 2040, Maßstab 20 µm).

4.4 Wirksamkeit von Antialgenstrategien an Baumaterialien

In Farben und Putze eingebrachte Biozide können Algenbewuchs an Baumaterialien verhindern. Dennoch ist der Einsatz chemischer Antialgenstrategien nicht ohne Gefahren für die Umwelt, da die Wirkstoffe mit dem Regen ausgewaschen werden und in Böden und Gewässer gelangen können, wo sie dort lebende Organismen schädigen. In den letzten Jahren wurden zahlreiche umweltgefährdende Wirkstoffe verboten und neue umweltschonendere Mittel entwickelt. Diese zeichnen sich nicht mehr durch möglichst hohe Persistenz und breites Wirkungsspektrum, sondern Stabilität, an die Anwendung angepasste Wirkungsspektren und geringe Toxizität sowie Ökotoxizität aus (Brill 1995). Die Neuentwicklung von Bioziden und deren Zulassung ist jedoch mit hohem finanziellem Aufwand verbunden. Daneben führt ein gesteigertes Umweltbewusstsein der Bevölkerung zu einer zunehmenden Ablehnung von biozid ausgestatteten Baumaterialien und zum Wunsch nach umweltfreundlichen Antialgenstrategien. Daher steht die Bekämpfung der Ursachen von Algenbewuchs an Baumaterialien im Fokus des nachhaltigen Bewuchsschutzes.

Der wichtigste Faktor für das Wachstum von aeroterrestrischen Algen auf Oberflächen ist die Wasserverfügbarkeit. Die Stärke natürlicher Algenbiofilme korrelierte mit der Wasserspeicherkapazität des Substrates (Gladis & Schumann 2011a). Durch die Reduktion des verfügbaren Wassers, kann Algenwachstum gehemmt werden. An Gebäuden können häufig konstruktive Maßnahmen, wie Dachüberstände, die Feuchtigkeit an der Oberfläche reduzieren. Durch veränderte physikalische Oberflächeneigenschaften sollen Algenadhäsion und -wachstum reduziert werden. Hydrophobierende Beschichtungen sind wasserabweisend, das Beispiel des Lotuseffektes zeigt jedoch die Schwächen dieser Technologie. Beim Lotuseffekt wird die Benetzbarkeit der Oberfläche reduziert und eine selbstreinigende Oberfläche entsteht (Solga et al. 2007). Während starker Regen tatsächlich dazu führt, dass Wassertropfen schnell von der Oberfläche ablaufen, dabei Schmutzpartikel mitreißen und die Oberfläche säubern, sind kleine Tropfen, wie sie etwa bei Tau entstehen, zu leicht, um abzulaufen. Unter den kleinen Wassertropfen werden Algen mit ausreichend Feuchtigkeit versorgt und bilden Biofilme, die sich später weiter ausbreiten können. Trocknen die Wassertropfen an der Fassade, hinterlassen sie außerdem oft auffällige Schmutzränder (Müller-Rochholz & Recker 2008). Der Lotuseffekt basiert auf einer sehr feinen Strukturierung der Oberfläche. Diese Struktur kann an Baumaterialien,

z.B. durch natürliche Erosion, schnell zerstört werden, wodurch der hydrophobierende Effekt verloren geht. An hydrophilen Oberflächen breitet sich das Wasser dagegen stark aus und der entstehende Wasserfilm trocknet schneller ab. Algenzellen und Schmutz aus dem Regenwasser werden gleichmäßig verteilt und sichtbare „*Hot Spots*" vermieden (Messal 2008). Dadurch sind entstehende Biofilmmuster weniger auffällig. Daneben ist die Adhäsion der Algen auf hydrophilen Oberflächen erschwert. Dennoch blieben auch diese Oberflächen nicht algenfrei (Messal 2008). Materialien mit einer geringen offenen Porenweite reduzierten die Menge der für die Algen verfügbaren Feuchtigkeit und waren weniger stark bewachsen (Gladis & Schumann 2011a). Dunkle Oberflächen absorbieren Sonneneinstrahlung stärker, sind dadurch um bis zu 50°C wärmer als die Umgebungsluft und trocknen schneller ab (Berdahl et al. 2008). Auf schwarzen Ziegel bildeten sich nur vereinzelt, an geschützten Kanten, Algenbiofilme, die durch die Materialfarbe auch noch kaum sichtbar waren (Gladis & Schumann 2011a).

Die Photokatalyse stellt eine Kombination aus physikalischer und chemischer Antialgenstrategie dar. Zum einen sind die aktivierten Oberflächen hydrophil, zum anderen wirken sie chemisch durch die Bildung von hochreaktiven Hydroxylradikalen. Gegen Bakterien auf Oberflächen waren photokatalytisch aktive Materialien wirksam (Kühn et al. 2003, Kiwi & Nadtochenko 2005).

Ein Ziel dieser Arbeit war es, zu prüfen, ob diese Ergebnisse auch auf Bewuchs durch aeroterrestrische Algen an Baumaterialien übertragbar sind. Laboruntersuchungen wiesen eine schädigende Wirkung der Photokatalyse auf verschiedene Zellfunktionen aeroterrestrischer Grünalgen nach (Gladis et al. 2010). Diese Algen waren jedoch so gut mit Wasser versorgt, dass sie wahrscheinlich keine Biofilme bildeten. Eine Wirkung auf Algenbiofilme konnte weder im Labor noch in einer mehrjährigen Freibewitterung nachgewiesen werden (Gladis & Schumann 2011a, Gladis & Schumann 2011b). Die in anderen Studien nachgewiesene Wirksamkeit gegen Bakterien kann aufgrund der Besonderheiten aeroterrestrischer Algen und ihres Habitats nicht auf Algenbiofilme übertragen werden:

(1) Der Wirkmechanismus der photokatalytischen Inaktivierung von Bakterien ist die Zerstörung der Zellmembranen durch Hydroxylradikale oder Elektronen-Loch-Paare an der Oberfläche des Photokatalysators (Kühn et al. 2003). Dabei wurden vor allem die Phospholipide der Zellmembranen peroxidiert (Maness et al. 1999). Erst danach wurden intrazelluläre Zellkomponenten und -funktionen angegriffen. Dazu

zählen die Oxidation von Coenzym A und DNA sowie die Hemmung der Enzymaktivität und der Zellrespiration (Matsunaga et al. 1985, Maness 1999, Dalrymple et al. 2010). Der erste Schritt der Membranzerstörung lief relativ langsam ab. Sind die Membranen jedoch einmal aufgebrochen, erfolgten intrazelluläre Schädigungen, die zu einer schnellen Inaktivierung der Bakterien führten (Benabbou et al. 2007). Kühn et al. (2003) beobachteten eine zunehmende Toleranz von Mikroorganismen gegenüber photokatalytisch gebildeten Radikalen mit steigender Komplexizität der Zellmembranen. Grünalgen, zu denen die meisten aeroterrestrischen Algen gemäßigter Breiten gehören, besitzen als Zellumgrenzung neben der Zellmembran eine Zellwand aus einem stabilen Cellulosegerüst. Bakterien haben dagegen nur dünne Zellwände und können daher durch Radikale von außen leichter geschädigt werden (Madigan & Martinko 2009).

(2) Unter natürlichen Bedingungen bilden aeroterrestrische Algen eine schützende Matrix aus EPS, welche eine Barriere darstellt und den Kontakt von photokatalytisch gebildeten Radikalen und Zellen behindert (vgl. Kap. 4.2, Abb. 4.1 C). Nutzten die Algen für ihr Wachstum Wasserdampf, bildeten sie wahrscheinlich einen Biofilm und wurden durch Photokatalyse nicht gehemmt (Gladis & Schumann 2011b). Deckten sie dagegen ihren Wasserbedarf aus flüssigem Medium, welches in feuchten Filtern gespeichert war, bildeten die Algen vermutlich keine oder weniger EPS und wurden photokatalytisch inhibiert (Gladis et al. 2010, Abb. 4.5).

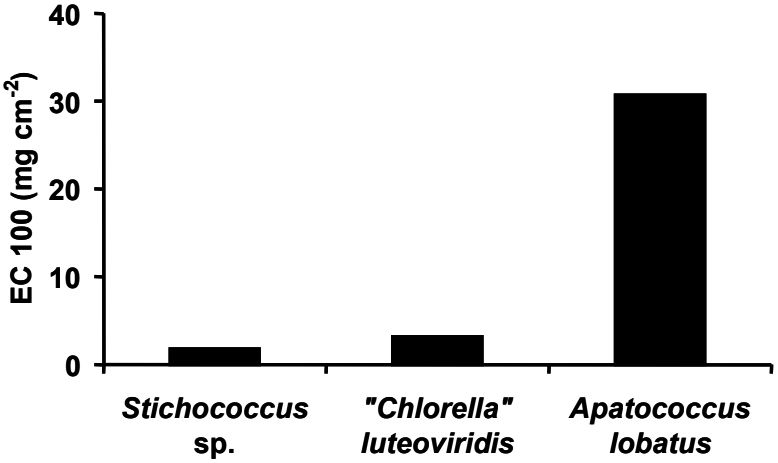

Abb. 4.5: Konzentration von photokatalytisch aktiven Zinkoxid-Nanopartikeln, die zu einer vollständigen Hemmung des Wachstums von aeroterrestrischen Algen, auf Glasfaserfiltern bei 100 % Luftfeuchte und täglicher Beregnung führten. EC 100 berechnet aus Dosis-Wirkungskurven (Gladis 2007).

(3) Algen in Regenwasser und Luft, welche sich an der Fassade anheften, liegen meist in größeren geschützten Aggregaten vor (Gorbushina 2007). Es ist anzunehmen, dass diese bei Anheftung die aktive Oberfläche beschatten und sie dadurch inaktivieren. Des Weiteren erreichen die kurzlebigen Radikale innenliegende Zellen nicht. Beim Wachstum in Suspension bildete nur noch die Alge *Apatococcus lobotus* die typischen Aggregate (Abb. 4.1 A). Diese Alge war dementsprechend auch deutlich toleranter als die übrigen, in Einzelzellen vorliegenden Arten (Gladis 2007, Abb. 4.5).

(4) Die photokatalytische Aktivität ist direkt abhängig von der Intensität der UV-Strahlung (Benabbou et al. 2007). Bisherige Studien zur Inaktivierung von Bakterien verwendeten meist deutlich höhere Strahlungsintensitäten als diese Arbeit, die sich an den natürlichen Bedingungen der meist beschatteten Standorte orientierte (z.B. Kiwi & Nadtochenko 2005: 40 W m^{-2} UVA). Vermutlich reichte die Strahlung in den hier gezeigten Laboruntersuchungen nicht aus, um die Photokatalyse ausreichend stark zu aktivieren. Im Freiland schwankt die natürliche Strahlung sowohl diurnal, saisonal als auch wetterbedingt. Es ist zu bezweifeln, dass die UV-Strahlung dauerhaft ausreichend hoch ist, um die Aktivität der Oberflächen aufrecht zu erhalten. So lag die UVA-Strahlung im Juli bei durchschnittlich 14,8 Wm^{-2}, während sie im Januar nur durchschnittlich 2,4 Wm^{-2} betrug (Standort Zingst 2002). Im Schatten reduziert sich die UV-Strahlung weiterhin um bis zu 90 % (Gies et al. 2007). Durch längere Phasen geringer oder fehlender photokatalytischer Aktivität, besonders in regenreichen und strahlungsarmen Monaten, wie z.B. April, kann sich auf Oberflächen ein Algenbiofilm bilden, welcher die aktive Oberfläche beschattet und eine spätere Aktivierung bei höherer Strahlung verhindert.

(5) An geschützten Ecken und Kanten oder in Oberflächenrissen, an welchen entweder kein Photokatalysator oder keine aktivierende UV-Strahlung vorhanden ist, kann ein mehrschichtiger Algenbewuchs entstehen, welcher sich auf die umgebende Fläche ausbreitet. Eine solche Ausbreitung der Algenbiofilme von Bewuchs begünstigenden auf Bewuchs hemmende Oberflächen wurde an den Kanten von glasierten Dachziegeln beobachtet (Gladis & Schumann 2011a).

Bisher gibt es keine umweltfreundliche Antialgenstrategie, die das Wachstum von Algen zuverlässig verhindern kann. Jedoch sind auch die Mechanismen der Besiedlung und Anheftung aeroterrestrischer Algen noch immer nicht aufgeklärt. Mit Hilfe dieses Wissens könnten Strategien entwickelt werden, um Algenbewuchs nachhaltig zu verhindern. Vorstellbare Entwicklungen sind Substanzen, welche die schützende

EPS angreifen oder, *Quorum Sensing*-Signalen ähnlich, die Adhäsion der Algen verhindern. In bakteriellen Biofilmen konkurrieren die Mikroorgansimen über auch gegen Algen wirksame antibiotische Sekundärmetabolite (Callow & Callow 2006). Hydrolytische Enzyme aus Bakterien lösen die Polymere der EPS und können möglicherweise die Bildung von Algenbiofilmen verhindern (Pettitt et al. 2004). Ein weiteres vielversprechendes Verfahren sind photovoltaisch beheizte Fassaden-beschichtungen. Diese könnten die Feuchtigkeitsbildung auf Oberflächen effizient verhindern. Die Kosten dafür werden jedoch in absehbarer Zeit zu hoch bleiben, als dass sich diese Anwendung in der Praxis durchsetzen wird (Scharf 2011). Schließlich könnten synergistische Effekte durch die Kombination chemischer Wirkstoffe und physikalischer Verfahren erzielt werden, um die Wirksamkeit einzelner Strategien zu erhöhen.

5 Zusammenfassung

Aeroterrestrische Algen bilden Biofilme auf natürlichen und anthropogenen Oberflächen und entwickelten zahlreiche Anpassungen an die extremen Bedingungen in ihrem Habitat. Auf Baumaterialien beeinträchtigen die Algenbiofilme durch ihre Verfärbungen nicht nur das Aussehen der Oberflächen, sondern können auch zu Schädigungen der Bausubstanz führen. Daher werden konstruktive, chemische und physikalische Antialgenstrategien zur Bewuchsbekämpfung eingesetzt. Potentiell umweltschädliche Biozide sollen jedoch durch neue funktionelle Oberflächenbeschichtungen, wie Photokatalyse, ersetzt werden. Um das Potential neuer Strategien zu bewerten, muss ihre Wirkung auf aeroterrestrische Algenbiofilme geprüft werden. Mit den etablierten Verfahren für die Zulassung chemischer Wirkstoffe lässt sich die Wirksamkeit von Antialgenstrategien jedoch nicht nachweisen.

In dieser Arbeit sollte zuerst die Frage beantwortet werden, wie die Wirksamkeit von Antialgenstrategien an Baumaterialien nachgewiesen werden kann. Die strukturellen und funktionellen Anpassungen der aeroterrestrischen Algen und ihre Lebensweise im Biofilm wurden charakterisiert und die Auswirkungen dieser Merkmale auf die Wirksamkeit von Antialgenstrategien dargelegt. Daraus konnten Schlussfolgerungen für die Durchführung von Wirksamkeitsnachweisen abgeleitet werden. Sowohl die Eigenschaften aeroterrestrischer Algen, die Bedingungen in ihrem Habitat als auch die Merkmale der Antialgenstrategien wurden berücksichtigt, um die Wirkung auf die Algen in Suspension und Biofilm sowie Labor und Freiland zu untersuchen. Daneben wurden verschiedene Vitalitätsparameter angewendet und ihre Eignung in Wirksamkeitsnachweisen diskutiert. Um die Wirkung von Antialgenstrategien, schwerpunktmäßig der Photokatalyse, auf aeroterrestrische Algenbiofilme im Labor zu bestimmen, wurde ein neues Nachweisverfahren entwickelt. Durch die Kombination von Wachstum und Membranpermeabilität war eine mögliche Wirksamkeit sensitiv nachweisbar. Das entwickelte Verfahren wurde als erster Wirksamkeitsnachweis für Antialgenstrategien zur Standardisierung vorgeschlagen.

Das zweite Ziel dieser Arbeit war die Untersuchung und Bewertung des Potentials von Antialgenstrategien für den Einsatz in der Praxis. Dabei wurden herkömmliche Biozide, Photokatalyse und physikalische Materialeigenschaften betrachtet. Während in Laboruntersuchungen gezeigt wurde, dass Photokatalyse die Vitalität aero-

terrestrischer Algen in Suspension inhibierte, konnte das Wachstum von Algen im Biofilm nicht unterdrückt werden. Eine mehrjährige Freibewitterung bestätigte die fehlende Wirksamkeit der Photokatalyse auf natürliche Algenbiofilme. Die Anpassungen an ihren extremen Lebensraum, wie z.B. die Bildung dicker Zellwände und EPS sowie das Wachstum in Aggregaten, schützten aeroterrestrische Algen vermutlich vor einer schädigenden Wirkung der photokatalytisch gebildeten Hydroxylradikale. Dagegen konnte durch reduzierte Wasserabsorptionskapazität der Oberflächen die Bildung von Algenbiofilmen im Freiland unterdrückt werden. Dennoch bildete sich unter geeigneten klimatischen Bedingungen auch auf diesen Oberflächen sichtbarer Algenbewuchs. Bisher gibt es kein Verfahren, dass Algenbiofilme wirksam und ohne Gefährdung der Umwelt verhindert.

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7 Anhang

7.1 Erklärungen

7.1.1 Anteilserklärung für Franziska Gladis

Gladis F, Eggert A, Karsten U, Schumann R. 2010. Prevention of biofilm growth on man-made surfaces: evaluation of antialgal activity of two biocides and photocatalytic nanoparticles. Biofouling

- Durchführung der praktischen Arbeiten
- Auswertung der experimentellen Daten
- Erstfassung und Bearbeitung des Manuskriptes

Gladis F, Schumann R. 2011. A suggested standardised method for testing photocatalytic inactivation of aeroterrestrial algal growth on TiO_2-coated glass. International Biodeterioration & Biodegradation

- Entwicklung des Messaufbaus
- Durchführung der praktischen Arbeiten
- Auswertung der experimentellen Daten
- Erstfassung und Bearbeitung des Manuskriptes

Gladis F, Schumann R. 2011. Influence of material properties and photocatalysis on phototrophic growth in multi-year weathering. International Biodeterioration & Biodegradation

- Durchführung der praktischen Arbeiten: Abmusterungen des Bewitterungsstandes in Zingst von 2007 bis 2009, abiotische Parameter der Ziegel
- Auswertung der experimentellen Daten
- Erstfassung und Bearbeitung des Manuskriptes

7.1.2 Selbständigkeitserklärung

Ich versichere hiermit an Eides statt, dass ich die vorliegende Arbeit selbständig angefertigt und ohne fremde Hilfe verfasst habe, keine außer den von mir angegebenen Hilfsmitteln und Quellen dazu verwendet habe und die den benutzten Werken inhaltlich und wörtlich entnommenen Stellen als solche kenntlich gemacht habe.

Franziska Gladis Rostock, den 24.06.2011

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7.3 Publikationsliste

Gladis F, Eggert A, Karsten U, Schumann R. 2010. Prevention of biofilm growth on man-made surfaces: evaluation of antialgal activity of two biocides and photocatalytic nanoparticles. Biofouling 26: 89-101.

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7.4 Tagungsbeiträge

Vorträge

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Gladis F, Schumann R. 2010. Algenfreie Fassaden durch Photokatalyse? 663. Kolloquium der DECHEMA: Mikrobielles Wachstum auf Fassaden und Dächern - Ursachen, Hintergründe und Bekämpfung. Frankfurt am Main 11. Februar 2010

Gladis F, Karsten U, Schumann R. 2008. Viability of phototrophic microorganisms - comparison of parameters. 11th International Conference on Applied Phycology. Galway (Ireland) 21.-27. Juni 2008.

Gladis F, Gustavs L, Schumann R, Karsten U. 2008. Microalgal growth on buildings - Ecophysiological performance and control. Meeting of the Specialist Groups “Desert Ecology” and “Experimental Ecology” of the Society of Ecology (GfÖ) in Cooperation with German Society of Limnology (DGL): Plant life in an extreme and changing environment. Tharandt 31. März-2. April 2008.

Gladis F, Schumann R, Plüschke S. 2008. Wirkung der Photokatalyse auf die Vitalität von Fassadenalgen. Workshop der Fraunhofer-Allianz Photokatalyse: Wirksamkeitsmesstechnik für Beschichtungen mit Nanomaterialien. Schmallenberg 12.-13. März 2008.

Gladis F. 2007. Wirkung von Bioziden und photokatalytischen Nanopartikeln auf die Vitalität von Fassadenalgen, DECHEMA-Arbeitsausschuss Mikrobielle Materialzerstörung und Materialschutz. Frankfurt am Main 4. Juli 2007.

Poster

Gladis F, Messal C, Schumann R, Karsten U. 2007. Efficiency of biocides against microalgal growth on facades. Botanikertagung der Deutschen Botanischen Gesellschaft e.V. (DBG). Hamburg 3.-7. September 2007.

Gladis F, Karsten U, Schumann R. 2007. Testing the vitality of phototrophic microorganisms. 7th European Workshop of European Society of Microalgal Biotechnology. Nuthetal 11.-13. Juni 2007.

Gladis F, Messal C, Schumann R, Karsten U. 2006. Wirksamkeit von Bioziden gegen Fassadenalgen. 11. Wissenschaftliche Tagung der Sektion Phykologie. Helgoland 28.-31. August 2006.

7.5 Lebenslauf

Persönliche Daten

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